Acta Medica Scandinavica

TABLE OF CONTENTS

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NORDISKT MEDICINSKT ARKIV — ACTA MEDICA SCANDINAVICA 100 YEARS

Acta Medica Scandinavica derives its origin from Nordiskt Medicinskt Arkiv (Nordic Medical Record) which was founded in 1869 and the journal can thus celebrate its centenary this year

The prime mover in the creation of the Record was Axel Key Professor of Pathological Anatomy at Karolinska Institutet in Stockholm Even before he became Professor he had evolved plans for starting a journal In his opinion the scientific literary work at the Institute was on too small a scale and did not me t up to the requirements which could and should be placed on it Nor undoubtedly did he overlook the fact that a heightened scientific activity would redound greatly to the reputation of the Institute so that it would not be overshadowed by the universities especially that at Uppsala with the medical faculty of which the Institute had at that time still to compete for equality The best way to stimulate his colleagues to scientific activity Key considered was to publish a new medical journal for original contributions within all branches of m dical science" as special organ for the Institute The incentive would then exist for one and all to provide matter for the journal and failure to do so would be felt as a neglect of duty The Institute as such could of course not publish and bear the responsibility for a new journal but this must b- done through the lecturers who voluntarily undertook to participate in the venture After certain difficulties *Medicinskt Arkiv* (Medical Record) was brought to birth and held its ground during the years 1863–1868 during which three volumes were published The editors were professors Troilius Rosander and Key one for the medical one for the surgical and one for the theoretical discipline

Although its circulation was very limited and its finances not particularly brilliant Key soon started to think of extending the journal into a central organ for the entire Scandinavian original literature in the medical field with contributions from all Nordic countries as well as reviews of the medical literature in the Scandinavian languages published in other journals" After he had found sympathy for his proposal from Peter L Panum in Denmark and Julius Nicolaysen in Norway and had also gained assurance of the support of Frithiof Holmgren in Uppsala Key presented the announcement of the new journal *Nordiskt Medicinskt Arkiv* (Nordic Medical Record) at the Scandinavian Meeting of Scientists in Kristiania (now Oslo) in 1868 This was at the session of the Medical Section there on July 10 Key pointed out that Scandinavian physici ins attended the Meetings of Scientists every fourth or every sixth year but in the intervals no collaboration took place Contact on the scientific plane was so poor that people knew better what was happening in Great Britain Germany or France than in the Scandinavian countries The scientific literature in our countries was also split among a large number of small journals and the language was incomprehensible to readers outside their frontiers To remedy these conditions the lecturers at Karolinska Institutet had decided to close down the journal Medicinskt Arkiv published by them and to bring out a new journal *Nordiskt Medicinskt Arkiv* which should have as its objective to be a central organ for the entire Nordic original medical literature Key thus presented the matter as already decided There followed a short discussion in the course of which certain misgivings were expressed It was stated among other things that an earlier journal *Nordisk Universitets Tidskrift* (Journal of the Nordic Universities) the object of which was to spread information concerning the Nordic universities and which was published from 1854 to 1856 alternately in Copenhagen Lund Kristiania and Uppsala, had had to be abandoned after two or three years

Axel Key initiator of our journal and editor 1869–1900

which was hardly encouraging for the idea of Nordic collaboration of the kind now planned In the event however Key received from all quarters appreciation and the promise of support The undertaking was thus launched

The editor was Axel Key assisted by editorial committees in Kristiania Copenhagen Lund Stockholm and Uppsala

The publication of the journal started in *1869* with four issues a year each comprising at least 12 sheets The first issue started with a paper by the Dane P L Panum Bidrag till Kunskaben om Misdannelsernes Oprindelse (Contribution to the knowledge concerning the origin of congenital deformities) Among other papers was one by A Tornblom Om den Listerska behandlingsmetoden av sår och abscesser (On Lister s method for the treatment of wounds and abscesses) the *first work on antiseptics to be published in* Sweden

There is no doubt that the effort was made from the start to maintain the journal on a high scientific level and with diversified contents and that this effort was successful

On the other hand the financial situation was a constant source of anxiety from the start The hospital system in the Nordic countries at that time was little developed the resources of the medical colleges and universities were very limited and the basis for an adequate circulation therefore virtually non-existent Nor was there any pharmaceutical industry to speak of No advertising revenue could be reckoned on nor any assistance from the State The journal ran at a loss every year which had to be covered by personal contributions from the editors which of course was not a stimulus to work

In due course certain grants were made from 1876 by the Swedish government and 1885 by the Danish government but they *altogether insufficient and the debts accumulated* Finally in the autumn of 1897 the situation came untenable for Key but was resolved in manner very favourable for him and the journal The entire debt consisted of unpaid printing bil from AB P A Norstedt & Soner in Stockho Norstedts now announced that they released Key from all liability and removed the debt from account as paid

The financial worries had thus been removed but only for a time The loss for the coming yea was so considerable and Key felt the financi responsibility so heavy that he could see on two alternatives—either the journal should

NORDISKT
MEDICINSKT ARKIV

FÖRSTA BANDET

STOCKHOLM

The first number of our journal published in 1869 then named *Nordiskt Medicinskt Arkiv*

discontinued after 1899 or radical changes and improvements must be made in the hope of increasing the circulation The possibility of obtaining a guarantee elsewhere to cover eventual losses should also be investigated

When these first changes proved to be without effect, Key decided in due course that the journal should be divided into two sections as suitable organs for the two recently established Nordic associations for internal medicine and for surgery He also proposed that papers should be written in German French or English and when written in a Scandinavian language should be accompanied by a resume in German

As from Vol 34 1901 accordingly the journal was issued in two sections one for surgery and one for internal medicine with separate subscription lists and with different even if collaborating editorial offices The editor for the medical section was C G Santesson assisted by a staff of some forty prominent scientists from Denmark, Finland Norway and Sweden The redactores were Israel Rosenthal Copenhagen J W Runeberg Helsingfors Peter F Holst Kristiania and C. G Santesson Stockholm It is the latter s memorial publication *Axel Key och Nordiskt Medicinskt Arkiv* (Axel Key and the Nordic Medical Record) Norstedts Stockholm 1932 which provides the basis for this account

To ensure the publication of the journal a guarantee association of Swedish physicians was formed The initiative came from Key but the practical work was taken over by Santesson as Key was already an ill man in 1894 and died on December 27 1901 The membership of the association changed from year to year being 43 at its highest, after 1920 only ten or so Altogether 74 persons were members during the life of the association When its activities ceased in 1925 it had made contributions virtually of the same



C G Santesson, editor 1901–1915



I Holmgren, editor 1916–1957

magnitude as the grants from the Swedish government during the same years Grants had also been made by the Norwegian and later by the Finnish government as also from certain funds in Denmark, Norway and Sweden the Classen Trust Fund Rask-Örsted Fund Nansen Fund the Letterstedt Society the Swedish Society for Medical Research and the Johansson Fund

Despite these various contributions the journal s situation was very precarious during the next few years

In due course Axel Key s son Einar Key joined the management as editor for the surgical section and I Holmgren (1916) for the medical Both were to play a very important part in the future development of the journal Chairman of the board of the Record Association was O Medin the well known polio researcher whose name has been linked with the disease

It soon became clear that it was undesirable to have two separate sections of the Record It was decided to publish two journals which after some discussion were given the neutral names of *Acta Chirurgica Scandinavica* and *Acta Medica Scandinavica* with subtitle *Nordiskt Medicinskt Arkiv* and under these titles the journals were published as from Volume 52 1919 the year after the end of the first world war As from Volume 85 1935 the subtitle was abandoned and our journal has since appeared under the name which readers of the later generation have become accustomed to *Acta Medica Scandinavica*

In this context it is perhaps worth mentioning that a medical journal with the name Acta now so characteristic of the Nordic journals was published in Scandinavia in the 18th century This I would undoubtedly have overlooked if the Abo editor of our journal Pekka Brummer had not mentioned it to me with a thought for

V I. LII

ACTA MEDICA
SCANDINAVICA

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SUB TITULO
NORDISKT MEDICINSKT ARKIV
CO M C XIX XEL KEY

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REDIGENDA CURAVIT
I HOLMGREN
Stockholm

The first number of volume 52 1919 with the new title *Acta Medica Scandinavica* but still with the old name as a subtitle

the coming centenary In 1783 a first volume of about 500 pages octavo was published in Uppsala Stockholm and Åbo with a title page reading ACTA MEDICORUM SVECICORUM seu SYLLOGE Observat et Casuum Rariorum in variis Medic partibus præsertim in HISTORIA NATURALI PRAXI MEDICA et CHIRURGIA Tomus I Cum XIX Tabb Æneis Upsaliæ Holmiæ & Aboæ Impens M SWEDERI Reg Acadd Bibliop MDCCLXXXIII This bulky volume which does not appear to have had a successor can hardly be of other than casual interest to the majority of our readers Those from the Nordic countries however may wish to hear something about the contents of this first attempt at a Nordic Acta journal and I will therefore give a brief account

After a one and a half page foreword by editor there follow bibliographies of the two known Swedish physicians Charles Linneaus rolus a Linne 1707–1778) and Nils Rosén v Rosenstein (Nicolaus Rosen von 1707–1773) the former reputed mostly as matician and reformer of botany the latter as pioneer in paediatrics Then follows Sectio containing reprints of seven dissertations during the period of Linnaeus successors in chair at Uppsala Carolus a Linne fil and C P Thunberg *Sectio Posterior comprises* medical papers by professors Sidren A Mu and Rosen von Rosenstein of Uppsala and hard Rosenblad and G Harmens of Lund by some physicians from the remainder of S den the best known of whom is probably Cas Trendelenburg

ACTA
MEDICORUM
SVECICORUM,
SEU
SYLLOGE
OBSERVATT ET CASUUM RARIORUM IN
VARIIS MEDIC. PARTIBUS, PRÆSERTIM IN
HISTORIA NATURALI,
PRAXI MEDICA ET
CHIRURGIA

TOMUS I

Cum XIX Tabb Æneis

UPSALIÆ HOLMIÆ, & ABOÆ
IMPENS. M SWEDERI, REG ACADD BIBLIOP
MDCCLXXXIII.

The first medical journal from the North with the name *Acta Medicorum Svecicorum* published in 1

As already indicated the journal was published both in Sweden and Finland (which at that time was united with Sweden) It was written entirely in Latin not in Swedish and was thus addressed to the entire scientific world which at that time had Latin as its lingua franca

I have found that a special edition was printed with Stockholm alone as place of publication Holmiae Typis Petri Hesselberg 1783 likewise written in Latin

But after this parenthesis let us return to our present journal

When the first number of *Acta Medica Scandinavica* was published in 1919 with I Holmgren as editor the leading redactores in the participating countries were H I Bing Copenhagen Peter F Holst and S B Laache Kristiania J O Schauman and T W Tallqvist Helsingfors and K Petren Lund

At the same time (since the 1915 annual meeting) Holmgren had succeeded O Medin as chairman of the Board of the Record Association When the time became ripe to split up the Record Association an association was formed for the publication of *Acta Medica Scandinavica* (1935) with Holmgren as chairman On his retirement in 1957 after 41 years of work he was succeeded as chairman by Nanna Svartz and as editor by the undersigned who am thus only the fourth in line during these hundred years

In the course of the years the circle has widened In 1937 collaboration was established with Holland which since then has had a special group of redactores And in 1939 Iceland was added with one redactor Altogether there are now 43 redactores who are more or less heavily engaged on the journal s work

Since the 1920s it has been a matter of course that the redactores in the Nordic countries should meet at the Nordic congresses for internal medicine There they have discussed various problems and made decisions on important questions of common interest These meetings have continued and grown and regular meetings of redactores now take place at every Nordic congress the latest of which was held this summer at Reykjavik in Iceland For me as editor these meetings have been extremely stimulating and appear to me to constitute the basis for our present fruitful collaboration

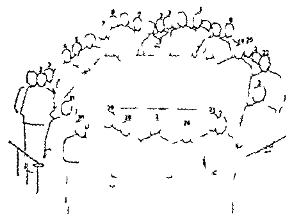
During the earlier years of *Acta Medica Scandinavica* the journal was issued according to the availability of suitable manuscripts in about three volumes a year divided into a number of fascicles which were published about every third week A volume might then contain fascicles which were published in two separate years As from Volume 165 1959 this unsatisfactory state of affairs was changed and one fascicle is published every month combined into two volumes a year corresponding in size to the three earlier volumes At the same time certain other improvements were made for instance the printing of two columns per page which makes the text easier to read

Apart from brief periods in 1922 and in 1938–1945 when the journal was printed by Mercators in Helsingfors and in 1946 when it was printed by Svenska Tryckeribolaget it has been printed throughout by AB P A Norstedt & Soner in Stockholm until last year when the printing was taken over by Almqvist & Wiksells Boktryckeri AB in Uppsala

Further improvements were now introduced which have given the journal an entirely new and more modern look The Summary was abolished and every paper instead starts with an Abstract Tabulation and type were changed so that the contents have become more compressed and the journal takes up less space on the shelf In order more easily to distinguish our journal from Acta chirurgica scandinavica we abandoned the red colour on the cover and went over to green which if one will may be denoted as an all clear signal to go ahead on the path that we have embarked upon

During the hundred years that have passed 184 volumes have been published in the regular series Since 1921 furthermore a series of supplements has been published containing doctoral theses congress proceedings monographies and other lengthy papers for which space could not otherwise be found So far 492 supplements have been issued Their expense has been borne by the authors or their patrons from funds or other sources and they have been distributed by the journal free of charge to subscribers who have thus had no expenses for them

It is naturally beyond the scope of a historical resumé of this kind to give even a brief account of the contents of the journal during the past century But a few random samples show that the



From the banquet at Stallmästaregården, Stockholm, June 24 1946 Two years earlier 75 years had passed since *Nordiska Medicinskt Arkiv* had started and 25 years since *Acta Medica Scandinavica* had been created in succession to the Arkiv but owing to the circumstances then existing (1944) it had not been possible to meet before to commemorate the celebration 1 S Hesser 2 J Sigurdsson Reykjavik 3 S Kallner 4 H Salvesen, Oslo 5 G Frumerie 6 N Svartz chairman since 1957 7 G Kahlmeter 8 G Hoglund 9 I Holmgren editor 1916–1957 10 P Formijne Amsterdam 11 S Bjorck 12 E Nyman 13 K Faber Copenhagen 14 O Hansen Oslo 15 J Mulder Groningen 16 E Jorpes 17 C Sonne Copenhagen 18 B Strandell editor since 1957 19 H. L Bing, Copenhagen 20 M Faber Copenhagen 21 C. Juhlin Dannfelt 22 F Saltzman, Helsingfors 23 K Warburg, Copenhagen 24 A. Gullbring 25 O Holsti Helsingfors 26 G Bergmark 27 Eggert Möller Copenhagen 28 W Kerppola Helsingfors 29 S Ingvar 30 E Salén 31 A Josefson

journal reflects fairly well the development that has taken place in internal medicine

From earlier volumes one immediately notes a paper by Armauer Hansen of Bergen Norway He discovered the bacillus leprae in 1873 If anyone were to make so brilliant a discovery in our days it would not be long before the editor received a manuscript with a request for imme

diate publication But not so with Armau r Hansen He submitted a report to the Medical Society in Kristiania in 1874 and thereafter seven years after his discovery had not thought of publishing anything when he was forced to do so through the action of foreign visitors on their return home Hansen then presented an account of his discovery entitled Bacillus leprae which was published in 1880 in Vol XII No 3 pp 1–10 This incident is reminiscent of William Withering who did not publish his discovery of digitalis until ten years later This it seems to me throws into the limelight the great change that has taken place as regards the publication of discoveries and observations

One may speak of an old Nordic school in the field of blood diseases It derives from the period around the turn of the century when the fundamental work on Bothriocephalus anaemia was being done in Finland with publications by J W Runeberg (1886) O Schauman (1894) and T W Tallqvist (1901) An important contribution was made by K Faber Denmark through his pioneering work on the relation between gastric function and anaemia (1909)—Faber s Syndrome Simple Achylic Anaemia Unfortunately these researchers pioneering achievements were not published in our journal other than possibly in the form of reviews in the review section for Nordic medical literature which the journal then had Only in later volumes do we find original contributions by some of these researchers

And S E Henschen s researches into the visual centre which also started at the turn of the century and led to the localization of the visual centre to the calcarine fissure where different parts of the retina can be projected onto the cortex of the brain are not mentioned at all in our journal until K Petren s obituary notice in Vol 65 1927

On the other hand H C Jacobæus published his new method of removing adherences in pneumothorax treatment of pulmonary tuberculosis in our journal (1914) under the title Endopleurale Operationen unter der Leitung des Thorakoskops

From the interwar period may be noted Robin Fåhræus classical work The Suspension stability of the Blood (1921) which is the subject of a special article by Erik Jorpes in this issue

A few years later (1931) came the work of the Finn E A von Willebrand Über hereditare Pseudohamophilie with an account of Einer Bluterfamilie auf den Alandinsel i e what is now called von Willebrand s disease

From the thirties derive too a series of papers by E Meulengracht of Copenhagen who charted the role played by different parts of the gastric region in pernicious anaemia

These are a few random samples from past times I have not on the other hand considered it necessary to mention any work by researchers still active in our circle as I presume they are well known to our readers

If we go on to later volumes however we find the development to have been that papers purely on clinical case histories which were common earlier have become increasingly rare They have made way for papers based on laboratory experiments with protein research and fat metabolism in particular in the centre of the stage and which provide contributions to the solution of the many puzzling problems still associated with the diseases which characterize our stressed age e g myocardial infarction and other vascular diseases In conjunction therewith investigations of the circulation and of arteriosclerosis have been intensified Many new discoveries have been made in the fields of blood diseases and protein metabolism and concerning collagenous diseases diabetes kidney diseases etc

In the past years therapeutic questions have very often been brought up for consideration One can easily follow in our journal the enormous development that has taken place in this field

Another characteristic feature is that the length of papers has been strictly cut in recent years Papers running to forty or fifty pages or more were previously common The aforementioned work by Fåhræus which admittedly consisted of four parts comprised no less than 228 pages The average length of paper in recent years has been eight pages and since the change made last year there has been a further reduction

We now publish some two hundred papers a year apart from some twenty supplements of varying size

The annual rise in the circulation must be seen as evidence that the journal is meeting the demands placed on it by its readers A larger circulation is also necessary Admittedly the journal works on a non profit basis but printing costs and

certain salaries have in recent years risen to a level such that the financial situation is not al together satisfactory although we look to the future with confidence

We anticipate a period of further specialization As a step in this direction The Scandinavian Jour nal of Infectious Diseases is to be published in close collaboration with our journal as from the beginning of this year

We remember with gratitude the decisive con tributions made on behalf of our journal by Axel Key and other far seeing men who are no longer with us

In conclusion I would address a word of warm thanks to our authors and all co workers for their collaboration and to our subscribers and readers for the interest they have shown in our journal *Acta Medica Scandinavica*

Birger Strandell

Acta med scand Vol 185 pp 9–12 1969

ENZYMES IN CELL FRACTIONS OF HUMAN LIVER BIOPSIES

Lactate dehydrogenase and glutamate oxalacetate transaminase in cell fractions of liver biopsies from patients with normal cirrhotic and fatty liver

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Abstract Liver biopsies from 31 patients with histologically normal liver from 14 patients with cirrhosis and from 17 patients with fatty liver have been homogenized and separated in mitochondrial and cytoplasmic fractions by centrifugation Lactate dehydrogenase (LDH) was determined in the cytoplasmic fraction and glutamate-oxalacetate transaminase (GOT) in both cytoplasmic and mitochondrial fractions. The activities were related to tyrosine-containing protein (Lowry) All activities showed a great variation from patient to patient in all groups but the mean values of cytoplasmic LDH and GOT were significantly depressed in cirrhotic livers and mitochondrial GOT was significantly elevated in fatty livers

Determinations of certain enzymes in the serum have been of great value in the diagnosis of liver diseases especially during active phases These enzymes have no known metabolic function in the serum but merely represent waste products from leaking or destroyed cells Their increase in the serum does not quantitatively reflect their deficiency in the liver while this deficiency presumably determines the degree of liver function impairment

The solution to this problem has appeared through the introduction of cytochemical methods which permit the quantitative determination of enzyme activity in cells (19 17) and subcellular fractions (20) from the small amount of tissue obtainable by needle biopsies of the liver in man In theory this opens large possibilities in the diagnosis and evaluation of liver diseases in practice however a number of difficulties regarding the interpretation of the results have to be overcome In the present work the problem is analysed by determination of two enzymes in liver biopsies from patients with normal and diseased livers

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MATERIAL AND METHODS

The material consists of 62 Menghini needle biopsies from adult patients They were divided into three groups according only to the histologic diagnosis the control group with no histologic abnormalities comprises 31 specimens 14 biopsies showed unequivocal cirrhosis and in 17 there was moderate to marked fatty infiltration

The syringe with which the biopsy was aspirated contained ice-cold 0 25 *M* sucrose The specimen was immediately divided into two parts one for histologic examination by routine procedures the other for cytochemical enzyme determination

Homogenization and fractionation was done by the method described earlier (2) Glutamate-oxalacetate transaminase (GOT) was determined (11) in both cytoplasmic and mitochondrial fractions Lactate dehydrogenase (LDH) usually was determined (10) in the cytoplasmic fraction alone as no significant activity was found in the mitochondrial fractions examined The protein concentration of the fractions was determined by a micro Lowry method devised by Holmgård (8) The enzyme activities are expressed per mg of tissue protein Usually the enzyme activity was determined immediately after the homogenization but occasionally the determination was postponed until the next day Stabilization with albumin and storage at 4 C prevented loss of activity for several days

The local anesthetic used for anesthetizing the integuments did not influence the activity determinations NADH oxydase and NAD ase activity was not found to a measurable degree The reproducibility of the fractionation

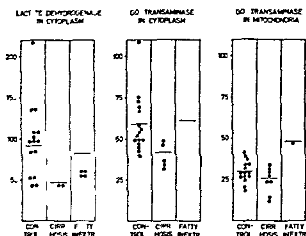


Fig 1 Enzyme activities in units per mg of protein tyrosine in subcellular fractions of liver cells obtained by needle biopsy from patients with histologically normal liver cirrhosis, and fatty infiltration

procedure and the determination was found to be satisfactory in experiments in which biopsies were divided into two parts and each part was analysed separately

RESULTS

The enzyme determinations are presented in Fig 1 it is seen that the scatter of the values is considerable and that there is a great overlap between the groups in all three determinations It appears however that the mean value of cytoplasmic LDH activity in cirrhosis is significantly lower than in controls ($p < 0.001$) and in patients with fatty liver ($p < 0.005$) The mean cytoplasmic GOT activity in the cirrhotics is also significantly lower than in controls ($p < 0.025$) In the group with fatty infiltration the mean mitochondrial activity of GOT was significantly higher than in the controls and the cirrhotics ($p < 0.005$)

DISCUSSION

Determination of liver cell enzymes is a promising approach to evaluation of liver function but is fraught with practical and theoretical difficulties (15) Even if the sensitivity specificity and accuracy of the analytical procedures must be further developed it is our impression that progress is most needed regarding the interpretation of the results for clinical purposes

The enzymes LDH and GOT were chosen for the present study because the elevation of these activities in the serum are generally recognized indicators of liver damage To clarify the problem raised the results had to be correlated with established criteria for liver disease It was assumed that a normal liver biopsy and the histologic diagnosis of cirrhosis and fatty liver would provide the most well-defined basis for classification of the results Yet the overlapping of the values is considerable and numerous factors may account for this

An important point in determining enzyme activities in tissues is the reference used The activity may be related to wet or dry weight of the tissue to nitrogen content, to RNA or DNA to the number of cells to the number of mitochondria etc The Lowry method for protein determination used as reference in the present work has the disadvantage that it does not measure all protein, as it mainly depends on the tyrosine content On the other hand this means that fibrotic tissue which contains little tyrosine has little influence on the result and the low values of cytoplasmic LDH activity in cirrhosis therefore cannot be explained in this way In fatty livers cell protein as a reference basis presumably results in higher activities than if the number of cells had been used because part of the cell is occupied by fat The best suited reference will thus depend on the question asked and possibly the best information would be obtained by using several references simultaneously

Another limitation of cytochemical enzyme determinations is that no information is obtained about the localization of the activity in the tissue Inflammatory cells in the liver may have a high enzyme content and thus will give a false picture of the state of the liver Histochemical methods on the other hand may localize the activity but yield poor quantitation Again the best solution may be a combination viz. of cytochemical and histochemical procedures It appears that contamination with blood does not influence the cytochemical determinations significantly (18)

A number of factors such as sex and age (9) starvation (6) thyreotoxicosis (12) and cortisone (5) are known to affect liver enzyme activities The variation in the present control material could not be related to any of these factors

In experimental liver damage in rats decreased activity of LDH and several other enzymes has been demonstrated histochemically (7) and in ex

perimental fatty liver in rabbits an increased LDH activity was found histochemically but not cytochemically (in relation to wet weight) (4)

Ryser et al (14) studied LDH and GOT activity in unfractionated homogenates of liver tissues from eight cirrhotic and pre-cirrhotic patients Both enzymes were reduced to about 50% of control values if wet weight was used as reference and to about 80% with protein tyrosine as the reference Schmidt and Schmidt (16) who studied patients with hepatitis found little change in the overall activity of LDH and GOT but the ratio mitochondrial/cytoplasmic GOT was reduced to about half Figueroa and Klotz (3) did not find any change in total-cell GOT (related to protein tyrosine) in fatty liver and cirrhosis whereas alcohol dehydrogenase was significantly lowered In a large group of patients with many different liver diseases Zelman and Wang (21) could demonstrate a rough correlation between the degree of necrosis of liver cells estimated histologically on the one hand and both tissue and serum transaminase activity on the other This relation was confirmed regarding patients with hepatitis but not in extrahepatic biliary obstruction by Rudkowski (13)

It appears that our observation of decreased cytoplasmic LDH and GOT in cirrhosis and increased mitochondrial GOT in fatty liver agree with most of the published results but a detailed comparison is of limited value due to differences in the clinical materials in the type of enzymes determined in the references used and in the analytical procedures

The main problem however especially from a clinical point of view is how to interpret the results in terms of liver function Does low activity of an enzyme indicate that the metabolic process in question is insufficient? Asada and Galambos (1) found no correlation between alcohol dehydrogenase activity in biopsies and elimination rate of ingested ethanol in alcoholic patients This is not unexpected since the activity in a biopsy may give a very inaccurate picture of the total enzyme content of the liver and furthermore it appears that the rate of metabolic processes—at least under normal conditions—is determined by factors other than the amount of enzyme available The enzyme activity determined in vitro may deviate greatly in both directions from the in vivo activity

This does not mean that enzyme determinations are useless for the evaluation of the state of the liver cells the cellular enzyme content may still be a valuable measure of the ability of the cell to produce and conserve its enzymes Many more enzymes must be investigated under well defined clinical conditions however before the significance of the method can be assessed

ACKNOWLEDGEMENTS

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ETIOLOGY OF AORTIC VALVULAR DISEASE

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Abstract On the basis of a clinical material comprising 222 patients with aortic valvular disease of whom 161 had been subjected to aortic valvular surgery the etiol ogy of aortic stenosis and insufficiency has been considered In this material 31 had congenital aortic stenos s. Previous rheumatic infection was found in 27% of the patients In the majority of the remaining patients aortic stenosis is thought to develop on the basis of congenital bicuspid aortic valves which is one of the most common congenital anomalies

Aortic insufficiency is most often combined with aortic stenosis and of the same etiology as aortic stenosis In patients with pure aortic insufficiency however bacterial endocarditis is at the present time the most common cause of the insufficiency Aortic dilatation with normal aortic valves was a common cause of aortic insuffic ency The dilatation was seen in rheumatoid spondylitis follow ing surgery for coarctation of the aorta, and in som cases was of unknown etiology Syphilit c aortitis was seen in only one patient

The etiology of aortic valvular disease has been much disputed The difference of opinion has mostly centered around the etiology of aortic stenosis some authors like Karsner and Koletsky (3) maintaining that aortic stenosis is almost al ways a result of a rheumatic infection while others are of the opinion that aortic stenosis is caused by a degenerative lesion of aortic valves Support for the latter view has come mostly from clinical considerations as is well known aortic valvular disease is mostly found in higher age groups the mean age of patients with aortic stenosis being 10–15 years higher than that of patients with mitral stenosis Aortic stenosis is preponderantly found in men usually in three times as many men as women while in mitral stenosis the sex rate is reversed four times as many women as men Furthermore a history of rheumatic fever is found only in 30 of pa tients with aortic stenosis as against 60 in mitral stenosis

In recent years there has been a changing pat tern in the etiology of aortic insufficiency For merly an antecedent syphilitic infection was found in 85% of these patients while at the present time syphilitic etiology is very rare in aortic insufficiency

It might be worth while to reconsider the etiol ogy of aortic valvular disease at the present time as these patients are now subjects for aortic valvular surgery A review of a material consist ing of patients subjected to aortic valve surgery might contribute to clarification of the etiology of the disease and especially contribute to a clarification of the disputed etiology of aortic stenosis

MATERIAL

The patient group used as the basis for this review consists of two clinical materials

I One consisting of 114 patients of all age groups, subjected to evaluation for aortic valve surgery during the years 1960 to 1965 (6)

II A material of 108 patients subjected to aortic valve replacement during the period 1965 to 1968 This mate rial comprises only adult patients

In the first material aortic valve surgery by a con servative method (debridement of the aortic valve) was carried out in 54 patients

The age and sex distribution of the two materials is presented in Table I As will be seen there are three times as many men as women in both materials. As ex pected the higher age groups are more strongly repre sented in the later material

The first material ma nly consisted of patien s with aortic stenosis as aortic insufficiency was not being operated on during that period A slight aortic insuffi ciency concomitant with aortic stenosis was found in 39% of that material. The insufficiency was usually insignificant and did not prevent a cons rvative operation, con sisting of division of fused cusps and remo al of calcif c deposits on aortic val es as thoroughly as poss ble

The later material shows the following distribution be tween pure aortic stenosis pure insuff ciency and com

Table I. *Age and sex distribution*

Age (years)

	0–9	10–19	20–29	30–39	40–49	50–59	> 60	
Material I								
Men	7	14	5	13	24	18	3	84
Women	4	5	3	1	7	7	3	30
								114
Material II								
Men	0	0	1	15	33	29	7	85
Women	0	0	1	2	6	9	4	23
								108

bined stenosis and insufficiency (Table II). As will be seen, there were only nine patients with pure aortic stenosis. Fifteen patients had pure aortic insufficiency and altogether 84 patients had combined aortic stenosis and insufficiency. In the latter group 20 patients also had mitral valvular disease.

The first material lends itself most easily to consideration of the etiology of aortic stenosis, while a review of the latter material may contribute to clarification of the etiology of aortic insufficiency.

RESULTS

Aortic stenosis

If one defines congenital aortic stenosis as a disease presenting symptoms before the age of ten years, there were 35 patients (31%) who had congenital aortic stenosis. A history of rheumatic infection (rheumatic fever, scarlet fever, chorea) was found in 31 patients, i.e. 27%. The etiology in the remaining 48 patients (42%) is unknown. The findings at operation in these patients might be of considerable importance. Of 54 patients subjected to surgery for aortic valvular stenosis, 33 had bicuspid aortic valves at the operation. Inspection of the aortic valves during surgery or even autopsy studies may fail to distinguish between congenital bicuspidity of the valves and fusion of one commissure caused by a previous rheumatic infection.

Table II. *108 cases of aortic valvular disease*

Aortic stenosis	9
Aortic insufficiency	15
Combined stenosis and insufficiency	64
Combined stenosis and insufficiency + mitral valve disease	20
	108

Bicuspid aortic valves are one of the most common congenital anomalies of the heart. The combined incidence reported by Bacon and Matthews (1) from various pathological materials covering the years 1918–1941 is 0.54% in a total of 28 441 autopsies. Most of the patients with bicuspid aortic valves go through life without signs or symptoms from the anomaly. In some of them, however, degeneration of the valves occurs with subsequent deposition of calcific material producing aortic valvular stenosis.

It is interesting to note that this concept is not a recent one, as it was suggested by Peacock (5) as early as 1858. Osler (4) in 1886 drew attention to the liability to subacute bacterial endocarditis in patients with bicuspid aortic valves. Studying pathological specimens collected from nine pathological museums in London in 1959, Bacon and Matthews (1) found 55 specimens of bicuspid aortic valves. Twenty-eight of these were certainly congenital. Some of the others were probably also of congenital origin. Thickening of the valve cusps increased with age, calcification appeared in the 5th decade and was found in all specimens aged more than 60 at the time of death. Seven specimens showed bacterial endocarditis, and in no less than 13 there was aortic stenosis which was calcific in all but one case. Their findings support the supposition that congenital bicuspidity of aortic valves is one of the most important etiologies of aortic valvular stenosis. Our findings of bicuspidity in 33 of 54 patients operated on for aortic valvular stenosis strongly support this view.

Mention should also be made of Monckeberg's view of a primary sclerocalcific form of aortic stenosis. This type would then occur in tricuspid

aortic valves It should be characterised by certain pathological differences from rheumatic aortic stenosis the calcification starting in the sinus pocket and at the base of the valve and especially affecting the fibrous layer of the valve cusps The calcification should further involve structures adjacent to the aortic cusps and sinus pocket There are no findings in the present material to confirm the view of Monckeberg We would presently consider it unlikely that a primary calcification of normal aortic valves can occur which would produce aortic stenosis of sufficient degree to cause severe symptoms

Aortic insufficiency

As will be seen from Table II most of the patients with aortic insufficiency 84 of 108 had concomitant aortic stenosis and 20 of them mitral valvular disease in addition Aortic insufficiency in these patients is brought about by fusion of commissures and calcific deposits producing rigid aortic valves which are unable to close during ventricular diastole The etiology in the patients with combined aortic stenosis and insufficiency has not been gone into in detail We feel that the etiology as in patients with aortic stenosis is partly congenital partly rheumatic and partly based on congenital bicuspid aortic valves

We shall center our discussion on the etiology of the 15 patients with pure aortic insufficiency As will be seen from Table III previous endocarditis was the most common etiology occurring in four patients Some of these patients had a previous known history of aortic valvular disease In some patients the valvular disease was unknown It is now well recognized from other materials that bacterial endocarditis is the most common cause of aortic insufficiency (2) The insufficiency is produced by destruction of the valve cusps by the bacterial endocarditis or by perforation of the cusps The history of these patients is characteristic as there is usually a rapidly downhill course following bacterial endocarditis The progression of cardiac symptoms may be evident even during the febrile stage of the endocarditis but usually it becomes manifest during the first one or two months following endocarditis

Rheumatic disease of the aortic valves was found in only one patient He had a previous

Table III *Aortic insufficiency*

Bacterial endocarditis	4
Rheumatic	1
Rheumatoid spondylitis	3
Aortic dilatation	4
Operation for coarctation of the aorta	2
Syphilitic	1
	15

history of rheumatic fever and the valves removed at operation showed thickening of the cusps and rolling and shrinkage of the valve edges

In rheumatoid spondylitis aortic insufficiency may occur as was found in three of our patients This is a peculiar form of aortitis as the chronic inflammation occurs primarily at the basis of the cusps extending into the ascending aorta and producing weakening of aortic wall and dilatation of aortic ring with free aortic insufficiency On inspection there is a roughening of the inner surface of the ascending aorta resembling that found in syphilitic aortitis although it does not extend so widely into the ascending aorta There is no fusion of the commissures or cusps The findings on microscopic examination reveal a thinning of the media and definite fibrosis and thickening of the adventitia with infiltration of lymphocytes The vessels of the adventitia show a marked thickening with narrowing of the lumen and perivascular lymphocytic infiltration The main lesion is usually found in the lower third of the ascending aorta (Fig 1) Aortitis is not infrequently found in rheumatoid spondylitis We have personally observed it in two of 39 patients (7) and Toone et al (8) observed it in eight of 265 cases of rheumatoid spondylitis

A similar lesion of the ascending aorta may also be observed in Reiter s disease

In four patients in our series aortic dilatation of unknown cause was producing aortic insufficiency of severe degree The cause of aortic dilatation in these patients is unknown as artificial aortic valves were inserted The patients are still alive accordingly no pathological studies of the aorta have been carried out The aortic valves were normal but could not meet during diastole due to the severe dilatation of the valve ring

It is well known that in cases of medianecrosis of the ascending aorta a severe aortic insuffici



Fig 1 The aorta from the aneurysm Intima (*I*) not thickened The media (*M*) thin with cellular infiltration and necrosis near the adventitia. The adventitia (*A*) thick hyalinized and fibrotic with infiltration of lymphocytes. H E × 12

ency may be produced This is found in such diseases as Marfan's syndrome and in chronic dissecting aneurysm of the aorta No such instances are represented in this material

Two of our patients had previously been operated on for coarctation of the aorta Bicuspid aortic valves are known to occur in coarctation of the aorta and these may later give rise to aortic valvular disease Less well known is the occurrence of a medianecrosis in the ascending aorta in patients operated on for coarctation of the aorta Among 150 patients operated on for this anomaly we have seen two with rapidly developing aneurysm of the ascending aorta leading to rupture and sudden death In the two patients presented here aortic insufficiency was produced by dilatation of the aortic ring presumably due to weakening of the ascending aortic wall One of the patients had bicuspid aortic valves

As will be seen there was only one patient with syphilitic aortic regurgitation During the same period we have observed three further patients with syphilitic aortic regurgitation not requiring surgical treatment The fact that this etiology of aortic valvular disease is now rapidly disappearing is well known and is confirmed by our study

Apart from the causes of aortic insufficiency which are represented here we have seen two instances of aortic valvular disease concomitant with hypercholesterolemia (Muller Harbitz disease) In this disease cholesterol deposits are found extensively in coronary arteries In some of these patients cholesterol deposits may also be found on cardiac valves producing mitral or aortic valvular disease as was seen in five of Muller's patients (4) Our two patients so far have no severe symptoms They have therefore not been subjected to aortic valvular surgery

Aortic insufficiency may be a congenital anomaly accompanying ventricular septal defect, the defect being located in the upper portion of the ventricular septum Two such patients have been operated on in our hospital during the same period but they are not included in this study

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A 7 S GAMMAGLOBULIN COMPLETELY NEUTRALIZING THE RHEUMATOID FACTOR MACROGLOBULIN

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Abst act From antiserum to the Rheumatoid factor i e anti RF serum, an active principle completely neutralizing the Rheumatoid factor (RF) has been isolated A method described in this paper was used for the isolation. The RF neutralizing fraction which constitutes a part of the anti RF serum, was shown to be a 7 S globulin (γG or IgG) but of a special type Its main property is the capacity of neutralizing the RF This γG or IgG does not react with common anti-γG It would appear to be a new type of IgG which for the present is denominated IgR.

Some authors have expressed the opinion that common 7 S globulin (γG or IgG) might be used as an antigen for the Rheumatoid factor macroglobulin (RF) In this connection it has earlier been maintained that common γG (IgG) has the capacity of completely neutralizing the RF It seems now to be generally agreed however that native γG of the common type which constitutes the main part of so-called human gammaglobulin is not able to neutralize the RF unless some aggregated γG is present (1 6) It is likewise a well known fact that aggregated γG has a pronounced capacity for neutralizing the RF but still not completely (2 6) It has to be considered however that a test in which aggregated antigens or antibodies are involved does not form a true biological reaction

For several years Svartz has made different trials with the aim of finding a non aggregated substance completely neutralizing the RF The principal results of these investigations will be briefly reported in this paper

MATERIAL AND METHODS

An antibody to the RF was produced by injecting the RF isolated by us, into rabbits by the common method for producing antibodies An anti RF serum with high titre was usually obtained

The antiserum diluted 7 times with distilled water was precipitated in cold (+4 C 48 h) and centrifugated

The supernatant, which was concentrated to the original volume of the serum, was fractionated by chromatography on carboxymethylcellulose using phosphate buffers with pH 4 4 to 8 8 diluted with equal amounts of NaCl 0 9% It should be added that rechromatography was often needed

The sedimentation constant of the fractions was determined by ultracentrifugation All 7 S fractions obtained were tested by among other methods immunoelectrophoresis and by precipitation in different dilutions

The IgG (7 S) fractions, which reacted with RF on immunoelectrophoresis were tested by precipitation reactions in tubes. For this purpose isolated RF was mixed with different amounts of the IgG fractions and kept for 2 h at 37 C and thereafter overnight at room temperature Centrifugation. The supernatants were investigated for the presence of RF by immunoelectrophoresis precipitation test and Latex test. Negative tests were considered a sign of complete neutralization.

RESULTS AND CONCLUSIONS

As reported above an antiserum against the Rheumatoid factor abbreviated anti RF serum was produced by the common method for obtaining antibodies In precipitation tests between RF and anti RF titres up to 1 12 000 were obtained Chromatography was performed on the supernatant from the precipitation in cold of the anti RF serum Often but not always a 7 S fraction which completely neutralized the Rheumatoid factor (RF) could be isolated Fig 1 shows ultracentrifugation of this fraction As may be seen an isolated peak was obtained showing a sedimentation constant of 6 9 S Fig 2 demonstrates immunoelectrophoresis between this fraction and the Rheumatoid factor A single and

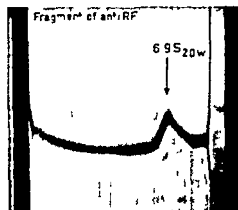


Fig 1 Ultracentrifugation of a fraction of anti RF serum, which completely neutralized the Rheumatoid factor (RF) The sedimentation constant of this fraction was 6 9 S

distinct precipitation line is demonstrable No precipitation was obtained between the anti RF fraction and anti γG (or γG) A definite but rather weak precipitation line was found between the anti RF fraction and RA serum and a slight one with normal human serum too but not with normal rabbit serum

It seems to be a question here of an earlier unknown type of 7 S gammaglobulin or in other words of a new IgG In order to lay stress upon the circumstance that this type of IgG has connection with the Rheumatoid factor and Rheumatoid Arthritis this 7 S gammaglobulin is for the present denominated IgR

Further investigations have to be made to show whether the IgR is found in antisera to the Rheumatoid like factor in other collagen diseases than Rheumatoid Arthritis particularly Systemic Lupus

Experimental studies in animals after injections of IgR will be reported in a following paper

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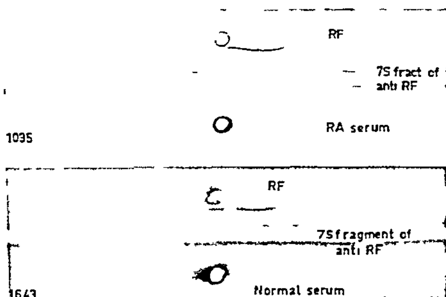


Fig 2 Immunoelectrophoresis between isolated Rheumatoid factor (RF) and an IgG (7 S) fraction of anti RF serum (for the present called IgR) There is also a precipitation line but less pronounced between Rheumatoid Arthritis serum and IgR.

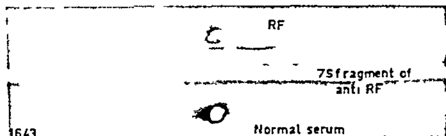


Fig 3 Immunoelectrophoresis showing precipitation between RF and IgR and also a slight precipitation between IgR and normal human serum.

Acta med scand Vol 185 pp 23–26 1969

ROBIN FÅHRÆUS AND THE DISCOVERY OF THE ERYTHROCYTE SEDIMENTATION TEST

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The clinical course in obstetrics is a favorite among Swedish medical students The long periods of waiting during the night hours provide an opportunity for thought In the delivery room the blood spattered student is forcibly impressed by the significance of blood as the life of the body especially since the life of the mother depends on the arrest of bleeding

It was in this environment while on duty at the Stockholm General Lying-In Hospital in 1915 that the medical student Robin Fåhræus (15 x 1888–18 ix 1968) observed a phenomenon which quite unknown to him had been described long before by John Hunter who in 1786 wrote as follows In all inflammatory dispositions the blood has an increased tendency to separate into its component parts the red globules being less uniformly diffused Fåhræus was equally unaware that the Dutchman van der Kolk, had observed this phenomenon in pregnancy and had described in 1820 how the blood from a pregnant woman separates into red flecks and flakes surrounded by absolutely clear plasma a phenomenon which does not occur in normal blood The fact is that in both illness and pregnancy some change in the blood causes the red cells to clump together like stacks of coins forming aggregates sometimes large enough to be seen with the naked eye The more extensive the clumping the more rapidly the red cells settle out of the uncoagulated blood a phenomenon which we now term an increase in the erythrocyte sedimentation rate

Fåhræus suggestion that this phenomenon might be useful in diagnosing illness resulted in a valuable clinical test which has the added advantage of being so simple to perform that it may be used under the most primitive conditions The blood made incoagulable by the addition of citrate is drawn up into narrow glass tubes which are then allowed to stand for one hour in a vertical position At the end of this period the level to which the red cells have settled out is measured That is all there is to it In healthy blood there will have been very little separation of cells and plasma in illness considerably more Immediately upon its introduction this technique was universally adopted as a diagnostic tool and soon was being performed almost as commonly as the measurement of body temperature

The presence of any inflammatory process acute or chronic is reflected in the sedimentation rate The test proved to be particularly valuable in the diagnosis of tuberculosis which was formerly a much greater problem than it is now

In reviewing the history of his discovery Fahræus had to turn to accounts written 75 and 150 years previously to find adequate earlier descriptions of the phenomenon and its relationship to disease states In the older literature he found a number of descriptions of what was called in Latin *crusta phlogistica* or *crusta inflammatoria*–in English buffy coat and in Swedish *spackhud* it was described as a white crust which formed on the top of the coagulum when blood was allowed to clot slowly in a tall glass vessel Its appearance on a sample from a blood letting was regarded as a sure sign of disease In England in the second half of the eighteenth century a young doctor Hewson (1739–1774) showed that the buffy coat consisted of what he called coagulable lymph and which Malpighi 100 years earlier called fibra thus fibrin

During the following years this phenomenon was studied by many other investigators includ ing the then greatest expert on blood the German Herman Nasse of Bonn In his book Das Blut published in 1836 (also published in R Wagner s Handworterbuch der Physiologie vol 1 pp 75–220 *Braunschweig 1842*) *Fåhræus found* most of his own observations described Even in Nasse s time it was recognized that clumping of the red blood cells must result in their more rapid sedi mentation just as the large drops in a shower of rain fall faster than the small droplets in a fog In blood with a rapid sedimentation rate the red cells settle out before coagulation occurs so that a white coagulum of fibrin alone is formed on the surface exactly as described by Hewson 75 years earlier The subsequent contraction of the fibrin clot produces the white crust which had attracted so much attention and been given so many names

Nasse regarded the clumping of the red cells as the result of a loss of electrical charge He made the empirical discovery that the clumping could be inhibited and the rate of sedimentation decreased if a salt such as ordinary kitchen salt or sodium bicarbonate was added to the blood sample It was of course impossible for him in 1836 to know anything about the ionic nature of the salts

The salt effect and the influence of the plasma proteins can be drastically demonstrated It some times happens in severe chronic infections that the panagglutination phenomenon is so strong that erythrocyte counting is impossible an awk ward situation in heavily anemic patients If in such a situation the erythrocytes are centrifuged down and the plasma replaced by an equal volume of physiological saline solution the agglutination is broken making the single erythrocytes visible

Nasse further attributed the clumping phenom enon not to the properties of the red cells them selves but to those of the blood plasma for when cells from samples of blood with a rapid sedi mentation rate and from samples with a slow rate were suspended in the same plasma their rate of settling-out was the same Nasse also made quan titative determinations of the fibrin forming ma terial of the blood according to the method of Berzelius and found that the increased sedimen tation rate in illness and pregnancy was accom panied by an increase in the level of the pre



Robin Fåhræus

cursor of fibrin the substance which Virchow was later to call fibrinogen

Fåhræus confirmed the correctness of Nasse s ideas about the effect of the properties of the plasma including the fibrinogen level and salt concentration on the stability of the blood cell suspension After Nasse s time these phenomena had been disregarded Virchow s cellular pathol ogy drew the attention of mid nineteenth cen tury medicine away from humoral pathology The latter field had been neglected for almost 100 years when Fåhræus rediscovered its significance and began to make use of it chemically

Fåhræus found that the levels of the high molecular weight proteins—fibrinogen and glo-bulins—are always increased in infections and certain other disease states and in pregnancy He used the albumin–globulin ratio as a measure of the change His doctoral thesis The Suspension Stability of the Blood was published in 1921 (Acta med scand 55) He had also published a paper three years earlier in Biochemische Zeit schrift (vol 89 p 355 1918) Über die Ursachen der verminderten Suspensionsstabilitat der Blut korperchen wahrend der Schwangerschaft He had even alerted physicians to the possibility of using the ophthalmoscope to observe the clumping tendency of the red cells in the blood vessels of the retina This parallels the course of the sedi mentation rate in disease

In the Chemistry Department at the Karolinska Institutet where Fåhræus did his analytical work and at the Seraphim Hospital across the street where Alf Westergren developed and refined the technique of measuring the sedimentation rate



Alf Westergren

fibrinogen and globulin were very familiar terms Fåhræus enthusiasm infected all around him with the possible exception of the brewery workers who once discovered the eccentric doctor in a white coat and with glasses pushed up on his forehead out in the yard stealing blood samples from their horses The blood of even a healthy horse has a very high sedimentation rate and the opportunity to get a blood sample from a brewery horse was an irresistible temptation for the discoverer of the sedimentation rate

Alf Westergren s Contribution

The practical details of the erythrocyte sedimentation test were elaborated by Alf Westergren (17 XII 1891–15 XII 1968) who at that time held an internship in medicine at the Seraphim Hospital the University Clinic of Karolinska Institutet. His name is as closely linked with the S R. test as that of Fahræus

The reliability of the test is completely dependent upon the way in which it is performed In taking the blood samples an admixture of ether or alcohol spoils the test Air bubbles in the syringe or in the needle do the same An essential feature is the diameter of the glass tubes which ought to be 2 5 mm and certainly not less Currents arising along the walls of narrow microtubes make the micro-sedimentation technique inapplicable Westergren paid particular attention to the choice of anticoagulant He recommended a 3 8 trisodium citrate solution to be mixed one part with 4 parts of blood according to which proportions he constructed a syringe to be used for the test The citrate like other salts causes a dispersion of the erythrocytes thereby somewhat retarding the rate of sedimentation The true sedimentation rate as observed in hemophilic blood is considerably higher than that seen when citrate is used Heparinized blood behaves like hemophilic blood in this respect Because of the low concentration of heparin 0 05–0 1‰ it does not exert any noticeable salt effect on the erythrocytes In comparing the different anticoagulants Westergren found the sedimentation reaction as performed with heparin as anticoagulant to be a less sensitive indicator of mild pathological changes in the blood than when citrate is used If heparinized blood is to be used for the sedimentation reaction it can be mixed with the 3 8 % citrate solution in the ordinary proportions In fact Westergren s original technique has proved to be superior to all later modifications even to the quick test performed with the glass tubes at an angle of 45

In this connection an unexpected result of Robin Fåhræus work deserves to be mentioned It gave the impetus to the invention of the angle centrifuge in 1927 by a young doctor Ragnar Lundgren at St Goran Hospital in Stockholm Lundgren experimented with a new technique using the sedimentation tubes set at an angle of 45 instead of vertically With the tube inclined the blood corpuscles sink along the lower side of the tube and the plasma flows over them in opposite directions Thus eddying and collisions among the blood cells which retard sedimentation are eliminated Westergren however soon demonstrated that the quick test of Lundgren has more drawbacks than advantages

But Lundgren in cooperation with a young engineer Gunnar Beckman went a step further and began applying the principle to the centrifugation technique By fixing the centrifuge tubes at an angle the sedimentation rate was greatly increased The angle centrifuge was developed by Beckman and supplied to the medical world by Vinkelcentrifug Wifug in Stockholm The number of tubes fixed in a head could be increased up to 60 and previous weighing of the tubes was eliminated This arrangement completely revolutionized the routine analytical procedures where centrifugations were necessary All over the world the angle centrifuge proved to be a technical aid of permanent value

However the idea for the design of the new

type of centrifuge originated not with an engi neer but with a young tuberculosis specialist who wanted to improve the S R. test Lundgren s mo dification of the technique of the sedimentation test however had more disadvantages than ad vantages and it never became popular the angle centrifuge on the other hand was a great success It ranks among Sweden s other achievements in this field the de Laval milk separator of 1878 Magnus Blix and Sven Hedin s hematocrite of the 1890 s and The Svedberg s ultracentrifuge from the 1920 s

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NORMAL AND LEUKEMIC CELL PRODUCTION IN MAN[1]

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Abstract It is conceivable that the effect of a tumor e.g leukemia, upon the host depends as much on the total production of tumor cells as on their proliferation rate For this reason an analysis of the human production rate of normal and leukemic cells was based on the in vivo ³H thymidine-labeling index variations found by Bond et al., and on thoracic duct drainage in patients with chronic lymphatic leukemia. Only three subjects were studied none of them quite healthy and several assumptions had to be made However the methods of analysis described may be of use

It is calculated that proerythroblasts divide most often—every five hours—and most quickly With increasing maturity the proliferation rate appears to decrease polychromatic erythroblasts divide only once in eight hours, and every division takes half an hour Of the former cells 51×10^{9} of the latter 2.0×10^{11} are formed daily In healthy persons the production may be somewhat smaller and times longer Assuming that some animal data on the distribution and life span of lymphocytes can be extrapolated to man, the very tentative figure that lymphocytes recirculate from blood to lymph nodes and back to blood about twice is obtained.

In normal persons some 4×10^{9} lymphocytes enter the blood daily via the thoracic duct whereas in leukemia some 4×10 enter Even if the leukemic lymphocytes did not recirculate at all and this latter figure equalled the real lymphocyte production in leukemia it would be smaller than the production of normal orthochromatic erythroblasts Even in these advanced cases of leukemia, accordingly the total production of tumor cells appears to be small when compared to the total production of normal bone marrow cells.

Thoracic duct drainage like extra-corporeal radiation removes some 10 lymphocytes daily or about one daily production and causes lymph nodes to decrease but unfortunately does not seem to improve the clinical condition of the patients.

It has been tempting to explain the expansion of tumors and the wasting of tumor patients on the basis of an increased proliferation of the tumor cells (28 29) However it is conceivable that the effect of the tumor upon the host depends on the total production of tumor cells as much as on their proliferation rate For this reason an attempt is made to study the total amount of cells produced daily in two severe cases of chronic lymphocytic leukemia and to compare the figure obtained to that for normal lymphocyte and bone marrow cell production For reasons given below the entire analysis is based on three patients only and the results are therefore to be regarded as preliminary The methods described however may be of use

NORMAL CELL PRODUCTION

Method of analysis

Normal erythroblast production was calculated on the basis of the variations in the fraction of labeled erythroblasts (labeling index, L.I) after an intravenous administration of ³H thymidine found in serial bone marrow samples from a single unconscious patient with apparently normal hematopoiesis and published by Bond et al. (4) This seems to be the only study in normal man of its kind

The analysis was based on a number of assumptions, the principal one of which was that changes in the L.I in a morphologically distinguishable maturation stage called here a compartment, occurs either when labelled cells in an earlier compartment mature i.e enter the compartment under study or when labeled cells within the compartment under study divide without an immediate change in their morphology A decrease in the L.I occurs when labeled cells mature and change their morphology i.e enter a following compartment.

The symbols used are described in Table I In addition to the conventional four generation phases (DNA synthesis phase Resting phase 1 Mitotic phase and Resting

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[1] The part of the present studies which is based on the work by Bond et al (4) was commenced during a visit to the Medical Research Center Brookhaven National Laboratory Upton N.Y., and reported preliminarily in 1961 (6)

Table I *Explanation of symbols*

I = number of cells ($T^{DNA}/T^3 \times C$) labeled during ^{3}H thymidine availability time ΔT

T (without index) = time immediately after ^{3}H thymidine injection

T^{DNA} T^M T^{R_1} T^{R_2} T^{R_3} – times for DNA synthesis mitosis and the three rest periods (R cf Method of analysis)

C = total number of cells in a morphologic compartment

T^1 = time from the part of DNA synthesis during which ^{3}H thymidine is available to completion of mitosis The approximation is made that for most of the labeled cells the availability time falls in the middle of the DNA synthesis period

T^2 = time from that part of DNA synthesis during which ^{3}H thymidine is available to transfer to next morphologic compartment

T^3 = total generation time in morphologic compartment

T^4 = *time from entering compartment to completion of mitosis*

T^5 = time from end of one homoplastic mitosis to end of next mitosis

K = fraction of the cells dividing in a compartment which will differentiate

X ($Y-1$) ($X-2$) ($X-3$) etc = order of compartments in a pipeline system

P B M O = proerythroblast basophilic medium or polychromatic and orthochromatic erythroblast compartments respectively

L I = labeling index

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phase 2) a fifth postmitotic phase (Resting phase 3) during which the morphologic transition from one compartment to another takes place was postulated These phases are illustrated in Fig 1 Certain necessary assumptions are listed in Table II and some assumptions often made consciously or subconsciously but not necessary in this analysis are listed in Table III The theoretical times for changes in the labeling index could thus be devised (Tables IV and V) as could the theoretical magnitude of these changes

Separate analyses were performed for the times at which the L I changes (Tables IV V and VII) and for the magnitude of these changes (Tables IV V and IX) A repetitive manual procedure was employed to fit the theoretical times and magnitudes thus obtained to those experimentally found by Bond et al (4) The standard deviation (S D) of each experimental point was calculated as for the binomial distribution

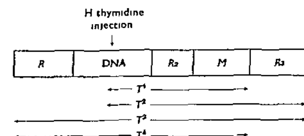


Fig 1 Illustration of symbols used for various phases of cell generation time

Table II *Assumptions made in present analysis*

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1 Sufficiently constant speed of DNA synthesis during DNA synthesis phase to permit all cells in that phase to become labeled
2 Initial compartment (P) self perpetuating However the flow or trickle of differentiating stem cells (19) into the P compartment would *not seem greatly to alter the* times found
3 No appreciable maturation without division
4 Availability time $t < T^{DNA} + T^M + T^{R_2}$ where t is that value for which $Ae^{-at} \approx 0$ if A is the initial serum concentration of ^{3}H thymidine and a is the rate of decrease in concentration
5 T^{R_3} or a corresponding time is spent even between two homoplastic mitoses

---

Table III *Common assumptions in bone marrow cell kinetics* not *made in present work*

---

1 Constant mitotic times for all cells in all compartments
2 Known type of mitosis (hetero or homo plastic) in all compartments
3 Borderlines between generations = morphological borderlines
4 Type of system known (random or pipeline An unsuccessful attempt was made to fit the experimental data to a random system (26))
5 Known relative times for some or all phases of the generation
6 *Assumptions about intracompartmental cell death*

$$S\,D = \sqrt{\frac{L\,I\,(1-L\,I)}{N}}$$

where the L I is expressed as a fraction and N denotes the number of cells counted in each compartment The S D is indicated by the T shaped lines on the figures

The relative compartment sizes i e the relative number of cells in each maturation stage and the mitotic indices in these stages were obtained from the data by Killmann et al (16) and are given in Table VI

Proerythroblasts (P)

Table VII shows the time limits obtained experimentally from Fig 2 and Table VIII the times employed in the analysis Slight deviations from the median were necessary in fitting the theoretical function to that experimentally found but all times used in the analysis were within the experimental limits It follows from Table VIII that the generation time $T_P^3 = 4\,7$ hours

Table *IX* shows the magnitude of the *L I changes* theoretically obtained as compared to those experimentally found from Fig 2

On the basis of these data (Tables VII and VIII) the following equations are obtained

Since $$I_P = \frac{T_P^{DNA}}{T_P^3} \quad \text{(Table I)}$$

Table IV *Labeled cells leaving an arbitrary compartment (x) in a pipeline system*

Time	No of labeled cells leaving
0	0
T^2	$K\ I_x$
$T^2+nT^5_x+T^R$	K_xI_x
$T^2_{(\ -1)}+nT^3_x$	$K\ K_{(\ -1)}I_{(\ \ 1)}$
$T_{(\ \ 1)}+nT^3_{(\ \ 1)}+nT^3_x$	$K\ K_{(\ \ 1)}I_{(\ -1)}$
$T^3_{(\ -\ )}+T^3_{(\ -1)}+nT^3_x$	$K_xK_{(x-1)}K_{(x\ \ 2)}I_{(x\ \ 2)}$
$T^3_{(\ \ 2)}+nT^3_{(\ \ \ )}+T_{(x-1)}+nT^3_x$	$K\ K_{(x\ \ 1)}K_{(x\ \ \ )}I_{(x\ \ 2)}$
etc	etc

Table V *Labeled cells entering an arbitrary compartment (x) in a pipeline system*

Time	No of labeled cells entering
0	0
ΔT	I_x
T^1	I
T^1+nT^3	$I\ (2-K\ )$
$T^3_{(\ -1)}$	$K_{(x\ \ 1)}I_{(x\ \ 1)}$
$T^4+T_{(\ \ 1)}$	$K_{(x\ \ 1)}I_{(x\ \ 1)}$
$T^2_{(\ \ 1)}+nT^3_{(\ \ 1)}$	$K_{(x-1)}I_{(\ \ 1)}$
$T^2_{(\ \ 1)}+nT^3_{(\ \ 1)}+(n-1)T^4_x$	$K_{(\ \ 1)}I_{(\ \ 1)}$
$T^2_{(\ \ 2)}+T^3_{(\ \ \ )}$	$K_{(\ \ 2)}K_{(x\ \ 1)}I_{(\ \ 2)}$
$T^2_{(\ \ 2)}+T^3_{(x-1)}+T^4$	$K_{(\ \ 2)}K_{(x\ \ 1)}I_{(\ \ 2)}$
$T^2_{(\ \ 2)}+T^3_{(\ \ 1)}+nT^3_{(\ -2)}$	$K_{(x\ \ \ )}K_{(x\ \ 1)}I_{(x\ \ 2)}$
$T^2_{(\ \ 2)}+T^3_{(x\ \ 1)}+nT^3_{(\ -2)}+(n-1)T^4_x$	$K_{(\ \ 2)}K_{(x\ \ 1)}I_{(\ \ 2)}$
$T^2_{(\ \ 3)}+T^3_{(\ -\ )}+T^3_{(\ \ 1)}$	$K_{(x\ \ 3)}K_{(x\ \ 2)}K_{(x-1)}I_{(x\ \ 3)}$
$T_{(\ \ 3)}+T^3_{(\ \ 2)}+T^3_{(\ \ 1)}+T^4_x$	$K_{(\ \ 3)}K_{(x\ \ 2)}K_{(\ -1)}I_{(\ \ 3)}$
$T_{(x\ \ 3)}+T^3_{(\ -\ )}+T^3_{(\ -1)}+nT^3_{(\ -3)}$	$K_{(x\ \ 3)}K_{(x-2)}K_{(\ -1)}I_{(\ \ 3)}$
$T^2_{(\ \ 3)}+T^3_{(\ -2)}+T^3_{(\ \ 1)}+nT_{(x-3)}+(n-1)T_x$	$K_{(x\ \ 3)}K_{(\ \ 2)}K_{(\ -1)}I_{(\ \ x}$
etc	etc

and $\frac{T_P^{DNA}}{4\ 7} = 0\ 47$ (Tables VIII and IX)

then $T_P^{DNA} = 2\ ?$

Since the mitotic index = 2 5 (Table VI)

$$0\ 025 = \frac{T_P^M}{4\ 7}$$

whence $T_P^M = 0\ 12$

Since $T_P^1 = 0\ 5\ T_P^{DNA} + T_P^M + T_P^R$ (*Table I*)

then $1\ 2 = 0\ 5 \times 2\ 2 + 0\ 1 + T_P^R$ (Table VI)

whence $T_P^R = 0$

Table VI *Relative numbers of total cells and mitoses in the compartments (16)*

Compartment	Relative no of cells	Mitotic index	Relative no of mitoses
Proerythroblasts (P)	0 495 B	0 0 53	0 01_5 B
Basophilic erythroblasts (B)	B	0 0494	0 0494 B
Medium (polychromatic) erythroblasts (M)	2.05 B	0 0565	0 116 B
Orthochromatic erythroblasts (O)	2.96 B	—	—
Total erythroblasts	6.5 B		

[a] Relative no of mitoses = (mitotic index) (relative no of cells)

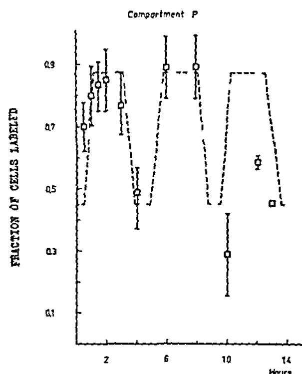


Fig 2 Labeling index variations experimental (□) and calculated ( ) in the proerythroblast compartment (P) For every experimental value ±1 standard deviation is indicated

Table VII *Experimental time limits obtained for proerythroblast compartment from Fig 2 (hours)*

Direction of L I change in P	Experimentally found time limits (Fig 2)
Labeled cells *enter* P	$0\ 5 < T_P^1 < 2\ 5$
	$4\ 5 < T_P^1 + T_P^3 < 6$
	$10 < T_P^1 + \ T_P^3 < 12$
Labeled cells *leave* P	$3 < T_P^2 < 4\ 5$
	$8 < T_P^2 + T_P^3 < 10$
	$1? < T_P^2 + 2T_P^3$

Table VIII *Solution of compartment P Times for changes in L I*

Labeled cells entering		Labeled cells leaving	
Theoretical times (Table V)	Times obtained from Fig 2	Theoretical times (Table IV)	Times obtained from Fig 2
T_P^1	1 2	T_P^2	3 2
$T_P^1 + T_P^3$	5 9	$T_P + T_P^3$	7 9
$T_P^1 + 2T_P^3$	10 4	$T_P^2 + ?T_P^3$	12 5

Proerythroblasts

R_1	DNA		R_3
0 4	2 2		2 0

$R_2 = 0$ ↑ ↑ $M = 0\ 1$

Generation t me = 4 7 h
0 5 of produ ed cells differentiate

Basophilic erythroblasts

R_1	DNA	M	R_3
1 9	3 0	0 4	2 0

$R_2 = 0$ ↑

Generation t me = 7 3 h
0 6 of produced cells d fferentiate
0 23 of produced cells d e

Polychromatic erythroblasts

R_1	DNA	M	R_3
1 5	2 0	0 45	4 1

Generation time = 8 h
0 56 of produced cells d fferentiate
0 025 of produced cells die

Fig 3 Erythroblast times as found in present analysis

Table IX *Magnitude of L I changes in compartment P*

Theoretical times	Total no of labeled cells in compartment	L I obtained from Fig 2
ΔT_P	I_P	?
T_P^1	$2I_P$	0 95
T_P^2	I_P	0 47
etc	etc	etc

Since $T_P^2 = 0\ 5\ T_P^{DNA} + T_P^R + T_P^M + T_P^R$ (Table I)

then $3\ 2 = 0\ 5 \times 2\ 2 + 0\ 0 + 0\ 1 + T_P^R$ (Table VIII)

whence $T_P^R = 2\ 0$

Since $T_P^3 = T_P^R + T_P^{DNA} + T_P^R + T_P^M + T_P^R$ (Table I)

then $4\ 7 = T_P^R + 2\ 2 + 0\ 0 + 0\ 1 + 1\ 0$ (Table VIII)

whence $T_P^R = 0\ 4$

In this manner the time parameters for the proerythroblast compartment can be calculated The agreement between calculated and experimental data is shown in Fig ? The result is summarized in Fig 3 It is seen that the generation time about 5 hours appears to be shorter

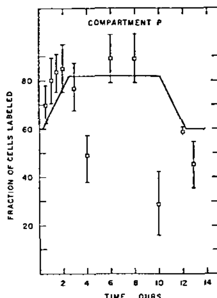


Fig 4 Alternative solution for proerythroblast pool This solution fits less well than that shown in Fig 2. □ experimental points —— calculated.

Table X *Basophilic erythroblast compartment (B) theoretical and experimental times*

Cells entering		Cells leaving	
Theoretical times	Times from Fig 5	Theoretical times	Times from Fig 5
T_B^1	1 8	T_B^2	3 9
T_P^3	3 2	$T_P + T_B^3$	10 5
$T_B^3 + T_B^1 = T_B^2 + T_B$	9 1		
$T_P + T_P^3$	7 9		
$T_P^2 + T_B^4$	8 4		

than previously suspected and that it seems to take an hour between the conclusion of mitosis and the appearance of the proerythroblast as a basophilic erythroblast.

The fact that longer times for the proerythroblast life span have been obtained earlier (summarized in (27)) made it necessary to test the fit of a longer life span to the data obtained in the proerythroblast compartment This attempt (Fig. 4) fitted less well than that shown in Fig. 2 and also seems to agree less well with some other data (77)

Basophilic erythroblasts (B)

The theoretical and experimental times are given in Table X and illustrated in Fig. 4 and the equations obtained by the same principle as for compartment P are as follows

From Table X

$$T_B^1 = 1\ 8 \qquad T_B^2 = 3\ 9$$

$$T_B^3 = 7\ 3 \qquad T_B^4 = 5.2$$

Since $MI_B = 0\ 05$ (Table VI)

$$T_B^M = 0\ 05\ T_B^3 = 0\ 36 \quad \text{(Table I)}$$

Since $I_B = 0\ 40$ (Table XI)

$$T_B^{DNA} = 0\ 4 \times T_B^3 = 2\ 92 \quad \text{(Table I)}$$

Since $T_B^1 = 0\ 5\ T_B^{DNA} + T_B^M + T_B^E$ (Table I and Fig 1)

$$1\ 8 = 1\ 49 + 0\ 36 + T_B^E \quad \text{(Table X)}$$

whence $T_B^E = 0$

Since $T_B^2 = 0\ 5\ T_B^{DNA} + T_B^F + T_B^M + T_B^E$

(Table I and Fig. 1)

$$3\ 9 = 1\ 5 + 0 + 0\ 36 + T_B^E \quad \text{(Table X)}$$

whence $2\ 0 = T_B^E$

Since $T_B^3 = T_B^E + T_B^{DNA} + T_B^E + T_B^M + T_B^E$

(Table I and Fig 1)

$$7\ 3 = T_B^E + 2\ 95 + 0 + 0\ 36 + 2\ 0 \quad \text{(Table X)}$$

whence $T_B^E \approx 1\ 9$

Since $T_B^4 = T_B^E + T_B^{DNA} + T_B^M + T_B^E$

(Table I and Fig 1)

$$5.2 = 1\ 9 + 2\ 95 + 0\ 36 + 0$$

which agrees well with the values in Table X

The magnitudes theoretical and experimental of the L I changes are seen in Table XI whence

$$I_B = 0\ 38 \quad \text{(Table XI)}$$

Since $K_P = 1$ (Fig 2 and assumptions)

and $I_P = 0\ 47\ C_P$ (Table IX)

$$K_P I_P = 0\ 47\ C_P$$

But $0\ 47\ C_P = 0\ 18\ C_B$ (Table XI)

or thus $C_P = 0\ 38\ C_B$

Table XI *Basophilic erythroblast compartment (B) magnitude of L I change*

Theoretical times	Total no of labeled cells in compartment	L I from Fig 4
ΔT_B	I_B	
T_B^1	$2I_B$	0 77
T_P^2	$2I_B + K_P I_P$	0 95
T_B^2	$2I_B + K_P I_P - K_B I_B$	0 50
$T_B + T_B^1$	$2I_B + K_P I_P - K_B I_B + I_B(2 - K_B - C_B)$	0 65
$T_P + T_P^3$	$2I_B + 2K_P I_P - K_B I_B + I_B(2 - K_B - C_B)$	0 83
$T_P + T_B^4$	$2I_B + 3K_P I_P - K_B I_B + I_B(2 - K_B - C_B)$	1 01
$T_P^2 + T_B^3$	$2I_B + 3K_P I_P - 2K_B I_B + I_B(2 - K_B - C_B)$	0 56

Table XII *Medium or polychromatic erythroblast compartment (M) times of L I changes*

Cells entering		Cells leaving	
Theoretical times	Times from Fig 6	Theoretical times	Times from Fig 6
ΔT_M	—	T_M^2	5 5
T_M^1	1 4	$T_B^2 + T_M^3$	11 9
T_B	3 9		
$T_M + T_M^1$	6 9		
$T_P^2 + T_B^3$	10 5		
$T_B + T_M^4$	10 9		

Since $K_B I_B = 0\ 45$ (Table XI)

and $I_B = 0\ 38$ (Table XI)

$$K_B = 1\ 18$$

Since $I_B(2 - K_B - C_B) = 0\ 15$ (Table XI)

$$0\ 38(2 - 1\ 18 - C_B) = 0\ 15$$

whence $0\ 33 - 0.38\ C_B = 0\ 15$

and $0\ 38\ C_B = 0\ 18$

and thus $C_B = 0\ 44$

The results obtained are summarized in Fig. 3 An alternative solution for B was also worked out, assuming that the decrease in LI_B at one hour was valid but could not be made to fit other data. The ratio C/C_B obtained from Table XI differs from the mean ratio given in Table VI probably because of the difference between individuals

Medium (polychromatic) erythroblast compartment (M) The times (Table XII) and magnitudes (Table XIII) of the L.I changes were obtained as above (Fig. 6)

The following equations are based mainly upon Table XII

$T_M^1 = 1\ 4$ (Table XII)

$T_M = 5\ 5$ (Table XII)

$T_M^3 = 8\ 0$ (Table X and XII)

$T_M^4 = 7\ 0$ (Table X and XII)

Since $I_M = 0\ 25$ (Table XIII)

$$T_M^{DNA} = 0\ \ 5 \times 8 = 2\ 0$$

Since $MI_M\ \ 0\ 057$ (Table VI)

$$T_M^M = 0\ 057 \times 8 = 0\ 45$$

Since $T_M^1 = 0\ 5\ T_M^{DNA} + T_M^M + T_M^R$ (Table I Fig 2)

whence $1\ 4 = 0\ 5 \times 2 + 0\ 45 + T_M^R$

and $T_M^R = 0$

Table XIII *Medium or polychromatic erythroblast compartment (M) magnitude of L I changes*

Theoretical times	Total no of labeled cells in compartment	L I obtained from Fig 5
ΔT_M	I_M	?
T_M^1	$2I_M$	0 52
T_B^4	$2I_M + K_B I_B$	0 72
T_M^2	$2I_M + K_B I_B - K_M I_M$	0 43
$T_M^2 + T_M^1$	$2I_M + K_B I_B + (2 - K_M - C_M)I_M - K_M I_M$	0 63
$T_P^2 + T_B^4$	$2I_M + K_B I_B + (2 - K_M - C_M)I_M - K_M I_M + K_P K_B I_P$	0 72
$T_B^2 + T_M^4$	$2I_M + 2K_B I_B + (2 - K_M - C_M)I_M - K_M I_M + K_P K_B I_P$	0 92
$T_B^2 + T_M^3$	$2I_M + 2K_B I_B + (2 - K_M - C_M)I_M - 2K_M I_M + K_P K_B I_P$	0 63

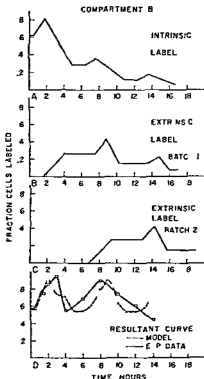


Fig 5 Times and L I changes in basophilic erythroblast (B) compartment. The top curve shows what the labeling index variations would be if no labeled cells entered from the proerythroblast compartment The middle curves show the entering labeled cells and the bottom one the complex picture obtained if all these curves are superimposed □—□ experimental value × × calculated value

Since $T_M = 0\,5\,T_M^D + T_M^R + T_M^M + T_M^R$ (Table I Fig 2)

whence $5\,5 = 0\,5 \times 2\,0 + 0 + 0\,45 + T_M^R$

and $T_M^R = 4\,05$

Since $T_M^3 = T_M^R + T_M^D{}^A + T_M^R + T_M^M + T_M^R$ (Table I Fig 2)

whence $8\,0 = T_M^R + 2\,0 + 0 + 0\,45 + 4\,05$

and $T_M^R = 1\,5$

The data obtained by analyzing the changes in L I magnitude (Table XIII) are used as follows

Since $I_M = 0\,26\,C_M$ (Table XIII)

and $I_B = 0\,38\,C_B$ (Table XI)

and $K_B = 1\,18$ (See above)

then $K_B I_B = 0\,20\,C_M$ (Cf Table VI)

The figures thus obtained from data given above agree well with line 3 in Table XIII

Since $K_M I_M = 0\,29\,C_M$ (Table XIII eq 4)

$K_M = 1\,1$

Since $(2\ \ K_M - C_M)\,I_M = 0\,20\,C_M$ (Table XIII)

$-0\,26\,C_M = -0\,014$

and $C_M = 0\,05$

Since
$$\left.\begin{aligned} C_P &= 0\,38\,C_B \\ K_P &= 1\,0 \\ K_B &= 1\,18 \\ C_B &= 0\,45\,C_M \end{aligned}\right\} \quad \text{(See above)}$$

and $I_P = 0\,47\,C_P$

$$K_P K_B I_P = 1 \times 1\,18 \times 0\,47 \times 0\,38 \times 0\,45\,C_M = 0\,096$$

which agrees with the values in Table XIII

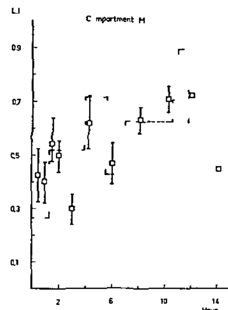


Fig 6 Times and L.I changes in medium or polychromatic (M) erythroblasts □ experimental values calculated values

Table XIV *Orthochromatic erythroblast compartment (O) times of L I changes*

Cells entering		Cells leaving	
Theoretical times	Times from Fig 7	Theoretical times	Times from Fig 2
T_M^2	5 5	$T_M^2 + T_O^3$	(?) 14 5
$T_B^2 + T_M^3$	11 9		
$T_P^2 + T_B + T_M^3$	18 5		

[a] Calculated

The results obtained for this M compartment are summarized in Fig 3 Fig 6 shows the degree of fit between the theoretical and the experimental data

Orthochromatic erythroblast compartment (O)

The labeling in this compartment originates in *M* since neither division nor DNA synthesis seem to occur here The times (Table XIV) and magnitudes (Table XV) of L I changes are as follows

$$T_O^3 = 9 \text{ hours} \qquad \text{(Table XIII)}$$

$$k_M I_M = 0\ 24\ C_O \qquad \text{(Table XIV)}$$

whence $1\ 12 \times 0\ ?6\ C_M = 0\ ?4\ C_O$

(See above and table XIII)

Thus $C_M = 0\ 82\ C_O$

Since no conventional phases in the cell cycle of these cells can be distinguished no summary is given in Fig 3 but their generation or sojourn time appears to be some 9 hours The degree of fit between the deducted and the experimental values is seen in Fig 7

Total erythroblast production

The patient weighed about 50 kg and can be assumed to have had 170×10^9 erythroblasts (27)

Since $C_P = 0\ 38\ C_B$

$C_B = 0\ 44\ C_M$

and $C_M = 0\ 82\ C_O$

then $C_P = 10 \times 10^9$

$C_B = 26 \times 10^9$

$C_M = 60 \times 10^9$

and $C_O = 73 \times 10^9$

Since generation times and thus the number of divisions per compartment per day are known as are fractions of produced cells entering subsequent compartments the daily production of the various cell types can be calculated

$B = 0\ 5 \times 2 \times 5\ 1 \times 10 \times 10^9 = 51 \times 10^9$ basophilic erythroblasts per day (produced in P compartment)

$M = 0\ 6 \times 2 \times 3\ 3 \times 26 \times 10^9 = 10? \times 10^9$ polychromatic erythroblasts per day

$O = 0\ 56 \times 2 \times 3\ 0 \times 59 \times 10^9 = 198 \times 10^9$ orthochromatic erythroblasts per day

(Table XIV)

In standard man the total daily erythrocyte production approximates 2 1 x 10 erythrocytes (19 27) which agrees reasonably well with the result above

Lymphocyte production

In dogs cats rabbits rats and calves (2 6 25 27-?8 31 35) the thoracic duct has been catheterized but because of the probable recirculation of lymphocytes from the blood via lymph nodes to blood the interpretation of the results thus obtained is uncertain It is true that studies of the daily lymphocyte replacement factor in rats based on the mitotic indices in lymph nodes after colchicine administration (?4) agreed well with that found by collecting thoracic duct lymph Similarly it has been maintained that only few (33) perhaps immunologically non-committed lymphocytes (15) may be recirculating and that some of the evidence for recirculation may be an artefact secondary to heparin (7 15)

On the other hand only some 1/40th of the lymphocytes in the rats body are found in the circulation (1 24) and ?9 to 5? of the lymphocytes leaving a lymph node seemed to be recirculating (14) Therefore it has been suggested that a great proportion of the lymphocytes (34) may be recirculating

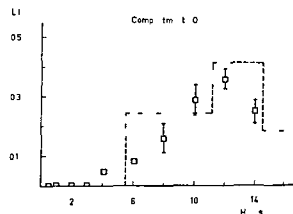


Fig 7 L I changes in the orthochromatic erythroblast compartment (0) □ experimental and deducted values

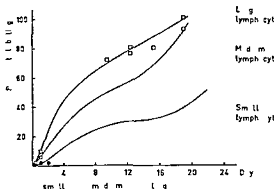


Fig 8 L.I changes in circulating rat lymphocytes after repeated H thymidine administration Ten rats (150 g) received 0 5 μc/g body weight every 12 h up to 21 *days and the labeling index was determined in brush* smears of lymph node (12 29) ● small ○ medium □ large

Two other pieces of information are available Cronkite (7) estimated that of the total number of lymphocytes entering the blood only one fourth to one sixth enter via the thoracic duct Finally there is information on the life span of lymphocytes which has been determined in rats by repeated infusion of H thymidine every 12 hours and by studying the time necessary to label all cells Results are shown in Fig 8 (12 29) Since it can be assumed that all new cells are labeled by this technique the time to label all cells should approach the life span of these cells. The same experiment but with a better technique has been performed by Brecher (0) with similar results suggesting that in the rat the life span of the small lymphocytes is at least of the same order of magnitude as that for the rat erythrocytes If one could extrapolate this would mean at least 100 days in man

Some attempts to calculate the total body lymphocyte number and production have been based on animal experiments (1 8 13 24) The total number is 2×10 per kg of body weight (1 4) or about 1.5×10 in a man. With an average life span of 100 days the daily production would approximate $1\ 5 \times 10^{1}$ which agrees well with Baserga s figures based upon mitotic counts and also with those calculated by Yoffey (33) and Fichtelius (13) after correction for the lower lymphocyte count in man (33)

On the basis of the discussion above and assuming the distribution data (1 24) and the information about the entering routes (7) to be valid in man the following is obtained

Total number of lymphocytes in the circulation (c) $\approx 5 \times 10^{9}$

Total number of lymphocytes in the body (b) $\approx 1\ 5 \quad 10^{2}$

Life span of small lymphocytes (l) = 100 days (12–13 20 29)

Daily production (p) in normal steady state $\approx 1\ 5 \times 10^{1}$

n = Mean number of times that each lymphocyte passes the lymph–blood barrier e g via thoracic duct = (mean life span)/(mean recirculation time)

d = Number of lymphocytes passing directly from lymph to blood daily = $5\,t$ (7)

t = Number of lymphocytes emptied into blood via the human thoracic duct i e 4×10^{9} (2 35)

But $\frac{t+d}{n} = p$ whence

$$p = \frac{6\,t}{n} \quad \text{and in healthy humans}$$

$$n = \frac{6 \times 4 \times 10^{9}}{1\ 5 \times 10^{\ 0}} = 1\ 6$$

Since the two assumptions given above are relatively *uncertain so is the figure thus obtained for n* which does however agree with some previous estimates (13 14)

LEUKEMIC LYMPHOCYTE PRODUCTION

A thoracic duct drainage was arranged in the following two patients by Franksson and Werner as previously described (2, 35) The patients were selected because of the severity of their disease and of their poor prognosis

Patient II H male 55 years old chronic lymphatic leukemia for two years general condition severely de*teriorated prior to operation, with thrombocytopenia* 400 000 lymphocytes mm circulating blood and hepato- and splenomegaly wasting and axillary cervical and inguinal lymphadenopathy (Fig 9) Thoracic duct drainage was maintained for 11 days The thoracic duct lymph was rich in lymphocytes (Fig 10) which were however frequently difficult to count because of clumping and aggregation The detailed results are seen in Table XVI Although an effect could be noted on peripheral lymph glands, which diminished distinctly (Fig 9) and became partially lymphocyte-depleted (Fig 11) on the number of circulating lymphocytes which was halved (Table XVI) and on the number of reticulocytes which increased (Table XVI) neither the platelets nor the total

Table XV *Orthochromatic erythroblast compartment (O) magnitude of L I changes*

Theoretical times	Total no of labeled cells in the compartment	L.I obtained from Fig
T_M^2	$K_M I_M$	0 24
$T_B^2 + T_M^2$	$K_M I_M + K_M K_B I_B$	0 42
$T_P + T_B^2 + T_M$	$K_M I_M + K_M K_B I_B + K_P K_M K_B I_P$	?
$T_M^2 + T_O^2$	$K_M K_B I_B + K_P K_M K_B I_P$	?



Fig 9 Axillary lymph glands in patient H H before (*top*) and after (*bottom*) thoracic duct drainage A distinct reduction in size is seen

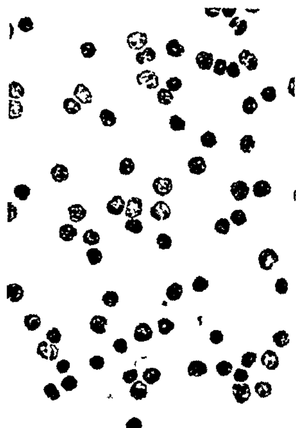


Fig 10 Smear from lymphocyte rich thoracic duct lymph showing some erythrocytes but mainly lymphocytes and lymphoblasts

body hemoglobin increased The two latter parameters may be considered the most relevant in judging the effect of leukemia treatment for it is not the number of leukemic cells which endangers the patient but the lack of normal cells i e the thrombocytopenia and the anemia

No decrease in splenic size could be noted nor could any decrease in the lymphocytic infiltration in the bone marrow be found The patient died in a general septicemia 23 days after the operation

Patient E A male aged 70 chronic lymphatic leukemia for one month with poor general condition 500 000 lymphocytes/mm of blood general lymphadenopathy (Fig 12) moderate spleno and hepatomegaly and lymphocytic lymphomatous infiltration of bone marrow The condition was complicated by previous hypertension cardiac enlargement and incipient decompensation

A thoracic duct drain was maintained for three days before it clogged A moderate decrease in lymph gland size and a moderate and transient rise in platelets but

Table XVI *Effect of thoracic duct drainage in chronic lymphatic leukemia*[a]

Patient	Blood vol (ml)	Total circulating no of lymphocytes	Total body hemoglobin[b] (g)	Platelets (/mm³)	Reticulocytes (‰)
H H	B 4500	B $2\ 4\times10^{12}$	B 340	B 80 000	B 1
	A 3500	A $1\ 3\ \ 10^{3}$	A 310	A 30 000	A 22
E A	B 7600	$3\ 8\times10^{3}$	B 610	A 45 000	B 0
	A 7600	$3\ 4\times10^{3}$	A 515	B 90 000	A 2

[a] B = before thoracic duct drainage A = after
[b] Determined with the carbon monoxide method of Sjostrand
[c] 2 weeks after operation reduced to original value



Fig 11 Photomicrograph of cervical lymph gland after thoracic duct drainage showing partial lymphocyte depletion.



Fig 1 Axillary lymph glands in patient E A before (*top*) and after (*bottom*) thoracic duct drainage A reduction in size can be distinguished

no change in the total number of circulating lymphocytes or the total body hemoglobin was noted (Table XVI) The bone marrow after operation was as full of lymphocytes as before

The patient died two months after the operation in cardiac decompensation with pulmonary edema and the post mortem examination showed multiple cerebral malacias

DISCUSSION

The value of the present calculations on both lymphocyte and erythroblast production is limited because of statistics only three persons were studied The model selected here for the analysis of erythroblast proliferation and maturation is merely an assumption—although it t hop d a plausible one (1)—and the solution found is probably not unique although none of the other solutions tested could be made to fit. It is hoped that the magnitude and complexity of the problem under study can b considered to justify these limitations Hazards of operating the leukemic patients and of giving radio-thymidin to controls made more extensive studies difficult.

The assumption of a fifth mitotic phase of morphologic transition (T^R) was made in order to explain the observed variations in the labeling indices The conceptual difficulties previously pointed out (10 19) for Osgood's asymmetric mitoses would also seem to become smaller with this assumption During T^P the cell would be available for the "inductor substances" as suggested by Lajtha (19) and one would not have to assume a switch from asymmetric to symmetric mitoses

Table XVII *Lymphocyte loss by thoracic duct drainage as compared to extracorporeal irradiation of blood*

Method	Reference	Species	No of lymphocytes	Multiple of total circulating lymphocytes
Extracorporeal radiation of blood	(7)	Calf	4×10^{9}/min	8–42/10 h
Thoracic duct outflow	(7)	Calf	5–25 × 10 /min or 7–36 × 10^{10}/day	1–10 day
	Present work	Man (leukemia)	1–6 × 10^{10}/day	0 01

The present data do not agree with Cronkite s (10) conclusion that immature erythroblasts have longer mitotic times than the mature ones but they do agree with the findings of a shorter time in the immature cells (30) and with the T_M^M of about 30 minutes found in dogs (23)

The present results regarding the generation times of the normal bone marrow cells between 5 and 10 hours have been called unconventional (32) and others have assumed considerably longer and more uniform generation times Studies of ^{3}H thymidine grain count halving times have given much longer times (10) and so have studies based on mitotic indices (16) The latter ulted in maximum compartment transit times $(\approx T_B^9)$ for basophilic erythroblasts of 95 hours (16 17) In one patient a T^{DNA} was found of no less than 12–36 hours (9) Whereas it may be difficult to reconcile such long times with a known production of about 2×10^{11} red cells daily the present data resulted in a production of $2 6 \times 10^{11}$ The patient studied may have had a decreased red cell life span and thus too high production Even if some of the times obtained are thus short the method of analysis may be valid

The times calculated here also agree well with those based on an entirely different type of calculation the erythrocyte production and the bone marrow differential count (27) They also agree with data calculated for dogs by Bond et al (5 22) and at least for the few cell types studied with some recent in vitro results (32) such as time lapse cinematography (3) Lajtha (19) assuming similar times for the later but longer times than here for the early erythroblasts found his model to yield a larger total erythroid cellularity and a lower mitotic index than found experimentally and Baserga (1) found that the total erythrocyte production became too low when he calculated on the basis of a one hour mitotic time These conclusions also support the present data Finally it is now admitted that clearly something is amiss with grain count diminution (10)

Nevertheless one must still reserve judgment on the results concerning normal bone marrow cell generation times The data on the total erythroblast production however being the result of two different methods one of which is independent of a selected model ought to be much less controversial For this reason and since it is quite conceivable that the effect of a tumor on the host depends as much on the total cell production as on the cellular proliferation rate the present attempt to compare total normal and tumor cell productions was made

The relation between the thoracic duct lymph flow and lymphocyte production has been reviewed The figures obtained for the human lymphocyte life span some 100 days and for recirculation about twice are partially based on animal experiments and thus quite tentative The

Table XVIII *Normal and leukemic cell production in man*[a]

Cell type	No of cells produced daily
Normal cells	
Basophilic erythroblasts (B)	0 51 × 10
Medium (polychromatic) erythroblasts (M)	1 03 × 10 ¹
Orthochromatic erythroblasts (O)	1 98 10^{11}
Lymphocytes (2 35)	1 5 × 10^{10}
Leukemic cells	
Patient H H	2 2 × 10^{11}[b]
Patient E A	0 5 10^{11}

[a] Erythroblast production calculated from ^{3}H thymidine labeling (4 26)
[b] Lymphocyte production calculated from thoracic duct output assuming recirculation twice

same applies to the figure for the total lympho cyte production being derived from those men tioned above

It is not known whether the recirculation and release of leukemic lymphocytes is comparable to those of their normal correspondents if it is the production in chronic lymphatic leukemia seems to be increased by a factor of 10 Nevertheless it does not seem to reach the production of normal orthochromatic erythroblasts (Table XVIII) This fact may be relevant when cytostatic therapy—chemical or physical—is discussed

The thoracic duct drainage seems to remove about as many lymphocytes daily from humans as extracorporeal radiation from calves (Table XVII) A similar relation between drainage and radiation has been described in rats (21) Both in normal and leukemic cows and in man a decrease mainly in the number of circulating lymphatic cells follows extracorporeal circula tion (11) Thoracic duct drainage in man on the other hand appears to affect mainly lymph glands Therapeutically unfortunately both at tempts to remove tumor cells appear equally un successful (18) in spite of the fact that a num ber of cells is removed daily which is comparable to the daily production

ACKNOWLEDGEMENT

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Addition in proof Generation times of the order of magnitude of erythroblasts in the present paper 5 hours have been reported for lymphocytes by Cronkite s group (Exp Cell Res 46 441 1967)

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THE INCIDENCE OF CARDIAC MALFORMATIONS IN GREENLANDIC ESKIMOS

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Abstract 757 children born in Godthåb Greenland in the years 1957–1964 were reexamined in 1966 Ten cer tain and four probable cases of cong n tal heart disease were diagnosed mostly cases of ventricular septal defect The incidence of 1 3–1 9% is the hi_hest registered in any ethnic group The average mother s age of the pa tients was 3? 1 years, the average mother s age of the total material 26 8 years This d fference is significant The patients average number in the sibship was 6 3 dif fering significantly from that of 3.2 in the total material The social standard of the families of the patients was low There is some indication that the incidence of cardiac malformations in the Gre nlandic population is decreasing This may be due either to improved social *cond tions or to a chan ing reproductive pattern with* relatively fewer children born to old mothers

The incidence of congenital heart disease seems to differ between various ethnic groups From existing data McKeown and Record (6) have concluded that congenital heart disease is more frequent in East Asia than in Europe whether this difference is due to environmental or genetic factors Direct comparisons between individual studies are however invalidated by differences with regard to diagnostic criteria and intensity of examination

Doctors practising in Greenland have been under the impression that congenital heart disease is very frequent among Greenlandic eskimos (4 11) but the sparse and widespread population has been ar obstacle to the accumulation of suffi cient data for statistical evaluation In the present study the incidence of congenital heart disease in an urban Greenlandic population has been estimated on the basis of a material comprising a total of 757 children born in Godthåb Green land in the years 1957–1964 and followed up in 1966–1967 Thus at the time of the follow up none were under one year old which has been thought to be of importance for the diagnostic accuracy

MATERIAL AND METHOD

The study has been confined to children born by Green landic mothers Some of the children especially those born out of wedlock had Danish fathers Children whose mothers had left Godthåb before the time of follow up ha e not been included A total of 16 stillbirths should by definition have been included in the material but as autopsies have not been performed in these cases still births have been disregarded in the incidence calcula tion This leaves a material of 757 live born children— 405 boys and 35_ girls

The follow up comprised several steps All children still living in Godthåb have been clinically examined by the authors The examination included careful ausculta tion Children ha ing moved to other districts in Green land have been examined by the local practitioners All health certificates have been reviewed as well as all records concern ng hospitalisations in Greenland or in Denmark The material as a whole has been checked against the Central Tuberculosis Registry of Greenland wh ch contains information on the annual X ray examina tion of thorax of the total population Finally the death certificates of those children who had died before the time of follow up ha e been reviewed In all children with conspicuous cardiac murmur ECGs in standard leads and chest roentgenograms in two projections ha e been taken

The collected data were considered incomplete in a total of 7 boys and 9 girls, in most cases because the children had been adopted by famil es in Denmark and Greenland with unknown place of res dence It is how e er most probable that they were healthy children as there has been no information on malformation or disease in the r birth records or in hospital files They ha e therefore been counted as non affected

RESULTS

Thirty four children had died before the time of follow up Among 13 who died during the first

month after birth one had died from autopsy verified cardiac malformation (case 1) In none of the rest had autopsy been performed but there were no indications that they had suffered from congenital heart disease

Congenital heart disease was diagnosed in 14 children nine boys and five girls In one case the diagnosis was based on autopsy (case 1) one child had been admitted to the University Hospital in Copenhagen for cardiac examination (case 8) and in all other cases the diagnosis was founded on clinical examination combined with ECG and X ray (cardiologist Ole Lindeneg M D has evaluated ECGs and roentgenograms) This of course has influenced the certainty of the diagnosis which in four cases must be considered with some reservation especially with regard to the type of defect (cases 11 12 13 14)

Ventricular septal defect was diagnosed in six cases pulmonary stenosis in three Three patients had either ventricular septal defect or pulmonary stenosis one patient either an atrial or a ventricular septal defect Finally the one autopsied patient had a complex defect with atresia of the pulmonary artery

CASE REPORTS

Case 1

Boy died 18 days old Mothers age 30 No 4 of six children Autopsy atresia of pulmonary artery stenosis of tricuspid valve persisting foramen ovale and ductus Botalli myocardial fibrosis of right ventricle

Case 2

Boy aged 7 Mothers age 26 No 4 of five children No symptoms acyanotic Systolic murmur grade 3–4 maximum at left sternal border ECG normal Chest roentgenogram heart diffusely enlarged (Ventricular septal defect?)

Case 3

Boy aged 6 Mothers age 27 No 2 of four children No symptoms acyanotic Systolic murmur grade 5–6 maximum at upper left sternal border ECG normal Chest roentgenogram heart diffusely enlarged (Ventricular septal defect?)

Case 4

Boy aged 5 Mothers age 42 No 12 of 13 children No symptoms acyanotic Systolic murmur grade 3–4 maximum at left sternal border ECG normal Chest roentgenogram heart diffusely enlarged (Ventricular septal defect?)

Case 5

Boy aged 2 Mothers age 30 No 3 of four children No symptoms acyanotic Systolic murmur grade 3–4 maximum at left sternal border ECG right axis deviation Chest roentgenogram heart moderately enlarged with elevated apex (Ventricular septal defect?)

Case 6

Boy aged 7 Mothers age 22 No 4 of seven children Acyanotic Dyspnoea on exertion Normal somatic and psychical development Systolic murmur grade 5–6 maximum at upper left sternal border ECG right axis deviation Chest roentgenogram heart diffusely enlarged prominence of main pulmonary artery segment (Ventricular septal defect? Pulmonary stenosis?)

Case 7

Boy aged 2 brother of no 6 Mothers age 27 No 7 of seven children No symptoms acyanotic Systolic murmur grade 3–4 at upper left sternal border ECG right axis deviation Chest roentgenogram heart diffusely enlarged prominence of main pulmonary artery segment (Ventricular septal defect? Pulmonary stenosis?)

Case 8

Girl aged 3 Mothers age 33 No 7 of seven children No symptoms acyanotic Heart sounds normal ECG right axis deviation Chest roentgenogram heart diffusely enlarged prominence of main pulmonary artery segment September 1967 admitted to Paediatric Department University Hospital Copenhagen Cardiac catheterization ECG and chest roentgenogram compatible with a diagnosis of pulmonary stenosis Cino angiocardiography however showed very small contractions of the left ventricle giving suspicion of subendocardial fibroelastosis

Case 9

Boy aged 6 Mothers age 36 No 6 of eight children No symptoms acyanotic Systolic murmur grade 3–4 maximum at apex ECG right bundle branch block Chest roentgenogram heart slightly enlarged (Atrial septal defect? Ventricular septal defect?)

Case 10

Boy aged 9 Mothers age 36 No 11 of 12 children No symptoms acyanotic Systolic murmur grade 5–6 maximum at left sternal border ECG coupled extra systoles Chest roentgenogram prominence of main pulmonary artery segment heart not enlarged (Ventricular septal defect?)

Case 11

Girl aged 7 Mothers age 34 No 8 of nine children No symptoms acyanotic Systolic murmur grade 5–6 maximum at upper left sternal border ECG normal Chest roentgenogram prominence of main pulmonary artery segment heart not enlarged (Pulmonary stenosis?)

Case 12

Girl aged 6 Mother's age 31 No 4 of four children No symptoms, acyanotic Systolic murmur grade 5-6 maximum at upper left sternal border ECG normal Chest roentgenogram slight prominence of main pulmonary artery segment (Pulmonary stenosis?)

Case 13

Girl aged 5 Mother's age 33 No 5 of six children Underweight and miserable Acyanotic Systolic murmur grade 5-6 maximum at apex Parents do not allow further examination (Ventricular septal defect?)

Case 14

Girl aged 9 Mother's age 4? No 11 of 13 children No symptoms, acyanotic Systolic murmur grade 5-6 maximum at left sternal border ECG normal Chest roentgenogram heart diffusely enlarged increased pulmonary vascular markings (Ventricular septal defect Pulmonary stenosis?)

Thus the accumulated incidence of congenital heart disease in this Greenlandic population can be estimated as between 1 3 ± 0 3 ° and 1 9 ± 0 5 In the latter figure the four doubtful cases have been included

COMMENTS

The incidence of congenital heart disease found in the present series seems to be high compared with the incidences reported in other studies

Gardiner & Keith 1951 (Canada) (2) 0 22 ° MacMahon et al 1953 (England) (5) 0 32 ° Richards et al 1955 (USA) (10) 0 83 Carlgren 1959 (Sweden) (1) 0 64% Morton 1962 (USA) (7) 0 53 % Shull & Neel 1965 (Japan) (13) 0 70 present study 1 3–1 9 °

This comparison of course must be considered with reservation as the materials differ with regard to diagnostic criteria and intensity of examination In these respects the present material seems to be most similar to the study of Shull and Neel (13) from Japan

With regard to the type of cardiac malformation comparisons are even more problematic For geographical reasons it has been difficult in the present material to establish a definite topical diagnosis In the Swedish study published by Carlgren (1) 0 25 of live born had died before the age of 7 from their congenital heart defect whereas in the present series only one child had died of this cause and in none of the rest was the condition life threatening at the time of examination Between one third and one fourth of the patients in the Swedish study had a diagnosis of ventricular septal defect in the present study it is about one half These findings seem to indicate that it is primarily the incidence of not severely invalidating heart disease and among these cases ventricular septal defect (and perhaps also pulmonary stenosis) which is elevated in the Greenlandic population

The material allows a few aetiological considerations The average age of mothers of patients with cardiac malformation is 32 1 years (S E 1 5) compared with the average age of mothers in the total population examined of 26 8 years (S E 0 2) The difference is significant at the one per cent level Accordingly the patients with congenital heart disease had relatively high numbers in order of birth on an average 6 3 differing significantly from the average number of the total material of 3 2 ($0\,01 > P > 0\,001$) The size of the families of the patients was on an average above that of the total material Thus 11 of the 14 patients had four siblings or more The same was the case in 388 of 757 children of the whole series This difference is significant at the five per cent level

The social standard of the patients is difficult to assess quantitatively but in all but two cases the families must be considered as definitely poor even according to Greenlandic standards

The present series is too small for subdivision but the fact that ten of our patients were found among 389 children born in 1957–1961 and only four among 368 children born in 1962–1964 may indicate that the incidence is decreasing In this connection the very fast improvement of social conditions in Greenland—and especially in Godthåb—during the last decennium must be taken into consideration Regular control during pregnancy has been introduced obstetric as well as social problems are taken care of and milk calcium and vitamins are freely distributed to all pregnant women In the same period the nutrition has changed much and the Greenlanders especially those in urban districts have more or less adopted the Danish dietary habits

No case of rubeolae or measles during pregnancy were reported in the present series Two patients belonged to the same sibship but beyond this it was not possible to obtain reliable family histories with regard to congenital heart disease

month after birth one had died from autopsy verified cardiac malformation (case 1) In none of the rest had autopsy been performed but there were no indications that they had suffered from congenital heart disease

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DEATHS FROM ASTHMA WITH SPECIAL REFERENCE TO THE LAST DRUG TREATMENT

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Abstract Fifty four patients who died from asthma in hospital in 1956-1967 were analysed retrogressively with special reference to the drug therapy immediately before death

Clinically sudden and unexpected death was as common as death which was attributed to asphyxia but at autopsy (45 patients) mucous bronchial obstruction was established in 37 cases.

During the last 24 hours of life 51 of the patients were given sympathomimetics 31 of them several sympathomimetics concomitantly and 21 in nebulised form One of the patients died during an adrenaline injection and one made liberal use of the orciprenaline aerosol she had brought with her to hospital

The xanthine derivative (glyphylline) was given during the last 24 hours to 50 patients The glyphylline dose was over 3 g 24 hours in eight cases The death of seven of them was regarded as clinically sudden and unexpected at least one patient had manifest symptoms of central nervous system stimulation prior to death Equally large glyphylline doses however were given to the control patients in status asthmaticus who survived

Polypharmacy seems to have been a probable iatrogenic factor influencing the mechanism of death in at least four patients of the total material Corticosteroids were not administered to four patients who had been managed for the last month prior to hospitalisation with corticosteroid Other iatrogenic factors influencing the mechanism of death seemed to be technical complications of respirator treatment in two cases and the possibility that respirator therapy was delayed

Sudden and unexpected death from asthma has been the subject of several studies (5 10 13 18 24 27 28 30) The majority of deaths from asthma are attributed to asphyxia Other contributory mechanisms have also been suggested e g infection psychic factors vagal reflex respiration-depressing drugs and acute cardiac death Although the toxicity of adrenaline and theophylline derivatives was realised early the joint effect and cardiotoxicity of sympathomimetics (17) has attracted increasing attention in the last two years This prompted us to undertake a retrospective clinical analysis of patients dying from asthma with special reference to the drug therapy and the clinical picture of the mechanism causing the fatality

MATERIAL

The present series consisted of 54 hospital patients admitted for asthma with fatal outcome (32 women and 22 men) All the fatalities from asthma in the Medical Clinic of Turku University and Paimio Sanatorium in 1955-1967 (until August 31) were included in the series (Fig 1) The patients mean age was 55 years (range 22-78) and the mortality peak fell between the ages 50 and 60 For the purpose of comparison patients of the same sex and age hospitalised for asthma were selected from the same years to serve as a control material

Fourteen of the patients who died from asthma were known to have had a family history of asthma or allergic diseases (18 of the controls) There were exogenous allergens in the history of 21 patients (14) and in 17 patients (13) infection was the factor that provoked asthmatic attacks Thirty-one patients (15) had received corticosteroid or ACTH therapy before admission to hospital

The onset of the asthma duration of its history and number of hospital admissions for asthma are given in Table I

STATUS ON ADMISSION TO HOSPITAL

The status at the time of the patient s admission was evaluated as severe in 28 (21 of the controls) fairly severe in 24 (6) and in only two patient (7) as mild Infections were encountered in 32 patients Many of the patients were severely dehydrated The blood pressure was elevated (over 160/90 mm Hg) in 18 cases and 25 patients had

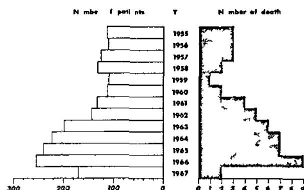


Fig 1 The annual number of patients hospitalised for asthma and the deaths from asthma (*until August 1967)

a pulse rate of over 100/min An ECG was taken from 40 patients during the last hospital stay It was within normal range in eight cases P pulmonale was established in 18 cases 14 patients had right ventricular strain and eight had other abnormalities

A chest roentgenogram was taken from 52 patients The finding was normal in 20 cases emphysema was seen in 17 and seven patients had fibrosis pleural adhesions parenchymal infiltration or some other pathologic condition Seven patients had cor pulmonale judging by the roentgenologic criteria and four had manifest congestive heart failure

Routine observation of the acid base balance has been practised at our hospitals only since 1963 The capillary blood Astrup values were determined in 14 patients an average of 17 hours before death PCO was normal (under 45 mm Hg) in one case 46–60 mm Hg in four 61–90

Table I *The duration of asthma and number of admissions for 54 patients who died from asthma and 54 control patients*

	Died	Controls
First symtoms of asthma after 40 years of age	30	31
Duration of the asthma		
Less than 2 y	4	10
2–5 y	9	11
5–15 y	17	18
More than 15 y	23	14
Unknown	1	1
No of admissions due to the asthma		
Not hospitalised earlier	11	7
1–3	13	24
4 or more	30	23

Table II *The duration of treatment in hospital before death (54 patients)*

Less than 24 h	13
1–2 days	14
3 7 days	11
More than 7 days	16

mm Hg in six and over 90 mm Hg in three cases The blood pH was normal in one patient 7 34–7 20 in eight patients 7 19–7 05 in four and *below 7 05 in one patient (6 96)*

TREATMENT

Treatment in hospital consisted of sympathomimetics (51 patients) glyphylline (50) antibiotics (40)—mostly tetracycline or penicillin—and corticosteroids or ACTH (38) The significance of hydration was not realised until the 1960s and only 36 patients of the present series were given *fluids parenterally the patients* also received ample fluids to drink Twenty four patients were given digitalis most of them without distinct signs of congestive heart failure Tracheostomy was performed on six patients and five of them (four of the controls) were kept in an Engstrom respirator One patient experienced relief from the acute phase but died after the termination of respirator treatment from pulmonary embolus which was established at autopsy Several patients obtained intermittent positive pressure breathing or oxygen through a nasal catheter

Table II shows the duration of hospital treatment The clinical evaluation of the mechanism of death is given in Table III together with the autopsy findings The histologic examination revealed a great many changes typical of asthma such as hypertrophy of the bronchial muscles thickening of the basement membrane and signs of infection In some cases the mucous plugging was so tenacious that it was possible to tear it off with forceps in a ramified chain resembling the bronchial tree Death from asphyxia did not seem to be as frequent a possible explanation clinically as in autopsy In six cases cardiac arrest definitely preceded respiratory standstill

DRUG THERAPY DURING THE LAST 24 HOURS

As can be seen from Table II 13 patients died within 24 hours of admission to hospital The

Table III *The mechanism of death*

Clinical impression	No of patients	Autopsy findings (45 patients)				No autopsy
		Mucous bronchial obstruction	Pulmonary embolism	Pneumonia	Uncertain mechanism	
Sudden and unexpected death	22	15	[illegible]	1	3	1
Asphyxia from suffocation in mucus	22	17	—	—	1	4
Clinically uncertain	10	5	—	—	1	4
Total number of patients	54	37	2	1	5	9

In this group asthma was the primary cause of death at autopsy for all the patients but the bronchi were no longer obstructed by mucus and there was also manifest congestive heart failure (4) severe emphysema ([illegible]) pneumonia ([illegible]) hemorrhagic pancreatitis (1) and mediastinal mesothelioma (1)

average time of treatment of these patients in hospital was 13 5 hours Table IV gives the drug therapy administered in hospital during the last 24 hours inclusive of the 13 patients mentioned above Sympathomimetics were given to nearly all the patients in the course of the last 24 hours Adrenaline was generally injected subcutaneously but some patients received intravenous infusion The 5 mg/24-hour dose was exceeded in only four cases Isoprenaline and orciprenaline were administered as aerosols or sublingually or perorally There was not a single case of acute aggravation of the patient s condition or death immediately after inhalation therapy The concomitant use of sympathomimetics was common The most usual combination was isoprenaline orciprenaline or ephedrine perorally as basic medication with an adrenaline injection or infusion One of the patients made liberal use in the last 24 hours of the orciprenaline aerosol that she had brought into hospital Aerosols were used a little more by the sudden death group than the other groups The theophylline derivative used was glyphylline the use of which has in

Table IV *Drug treatment during the last 24 hours before death (54 patients) and during the most severe 24 hours in 54 control patients who survived*

	Clinical impression			Total material	Controls
	Sudden death	Asphyxiated	Uncertain		
Sympathomimetic amines	22	19	10	51	54
Several sympathomimetic amines combined	15	11	5	31	41
Adrenaline					
Less than 2 mg	7	4	5	16	8
2 1 5 mg	6	8	—	14	7
More than 5 mg	1	2	1	4	—
Isoprenaline	9	7	1	17	17
Orciprenaline	14	7	2	23	17
Other sympathomimetic amines	6	4	7	17	31
Sympathomimetic amines in the form of aerosols	12	5	4	21	30
Glyphylline (i m or i v)	21	20	9	50	54
Less than 1 5 g	10	10	6	26	33
1 6–3 0 g	4	9	3	16	11
3 1–4 5 g	5	1	—	6	6
More than 4 5 g	2	—	—	2	4
Corticosteroids or ACTH	15	16	5	36	32
Parenteral fluids	15	15	6	36	38
Ataractica or barbiturates	4	7	4	15	23
Opiates	1	1	—	2	1

Table V *Iatrogenic factors possibly influencing the mechanism of death (number of cases among 54 deaths from asthma)*

Death during adrenaline injection	1
Polypharmacy — sudden cardiac arrest	4
Recent treatment with corticosteroids — not in hospital	4
Technical complication during respirator treatment	2
Delay in starting respirator treatment	?

creased considerably during the 1960s both in frequency and quantity Nearly half of the patients received this drug The maximum 24 hour dose recommended in the textbooks is 1 5 g The good tolerance of adults to theophylline derivatives and the exhaustion of aids available in an asthmatic crisis have tempted physicians to exceed this dose sometimes by a considerable margin The administration of glyphylline by intravenous infusion was often supplemented by intramuscular injections The maximum dose was exceeded more than twice in eight cases in two of them more than threefold Seven of these patients who were given glyphylline in abundance belonged clinically to the group of sudden and unexpected death

Table V shows the cases in which iatrogenic factors probably contributed to the mechanism of death One patient died while being injected with adrenaline and it is possible that the needle entered a vein We placed in the polypharmacy group only those patients whose death was clinically quite obviously cardiac arrest All these patients had been given massive combined therapy consisting of several sympathomimetics and at least double the maximum daily dose of glyphylline Of the patients who had received steroids either immediately before hospitalisation or in the course of one month before admission but not in hospital one died according to clinical evaluation from suffocation and three unexpectedly

DISCUSSION

It has been suggested that asthma developing late in life is particularly liable to end in death in status asthmaticus (10 15) In 30 of the 54 deaths reported here the disease began after 40 which tends to support this postulation Thirty of the patients had been hospitalised for asthma four or more times which is indicative of the danger of recurrent status asthmaticus (12)

Clinically unexpected death was a common finding in our material and this is in agreement with many earlier reports (5 10 13 26) Suffocation may occur extremely rapidly within a few minutes (30) The ostensibly great difference in the present series between the clinical assessment of the mechanism causing death and the autopsy finding may lie in extremely rapid death by asphyxia However situations that terminated clinically in manifest cardiac arrest without the dyspneic symptom point to the role of other mechanisms also in death from asthma

Acidosis diminishes the response to sympathomimetics (2) which is a temptation to use greater amounts of these drugs However acidosis also provokes arrhythmias (8) Recently it has been stressed that hypoxemia commonly persists despite considerable relief of airway obstruction by bronchodilator drugs (22 23) It is suggested that e g isoprenaline may intensify the ventilation perfusion disturbance in the lung and should therefore be used with caution in status asthmaticus (20)

Adrenaline has been blamed for sudden deaths from asthma (5 18) especially when overdoses are given However adrenaline disappears very quickly from the circulation Karki and Paasonen (14) showed in experimental animals that isoprenaline sensitised the systemic blood pressure to the effect of adrenaline and itself produced an increase in right ventricular pressure They pointed out the interacting effects of these drugs Excessive use of isoprenaline and other sympathomimetics especially in combinations or in pressurised aerosols has been suggested as a reason for cardiac arrest (9 19) The cardiotoxicity of isoprenaline and orciprenaline especially in myocardial failure and the enhancing effect of adrenaline toxicity has been emphasised (17) The clinical impression of the toxicity of sympathomimetics has also been criticised and it has been suggested that the administration of sympathomimetics alone or in combination leads to the development of tolerance rather than to increasing toxicity (1) Combined sympathomimetics were used in hospital on 31 of our patients who died from asthma but as many as 41 of the patients who survived also received this drug therapy Correlation between death and the ad

ministration of sympathomimetics is more dif ficult to establish but a connection is suspected in some cases The fatal cases received higher doses of adrenaline than the controls

The toxicity of xanthine derivatives is low But there are reports of deaths among adults suffering from heart and kidney disorders (3) and among children (24 29) Theophylline and noradrenaline increase synergically the inotropic effect on the cardiac muscle as well as phos phorylase activity (21) The interactions of sym pathomimetic amines and xanthine derivatives in vivo are perhaps more complicated but the pos sibility of increasing toxicity is presumable The dosage of glyphylline was exceptionally high in many instances and in one case the symptoms preceding the patient s cardiac arrest were typical of stimulation of the central nervous system The glyphylline dosage was more than 3 0 g/24 hours in eight cases and seven of these patients be longed to the fatalities evaluated clinically as sudden and unexpected Liberal glyphylline ad ministration in combination with sympathomi metic therapy may be assumed to have contri buted to the fatal termination in at least four cases Yet equally large glyphylline doses were given to the control patients in status asthmaticus who survived The explanation of this toleration of overdosage of glyphylline may have been that the drug was administered by slow intravenous infusion It has been contended that the cardio-toxicity of xanthine derivatives depends rather on the rate of administration than on the magni tude of the dose (3)

Impaired adrenocortical function has been re ported even in asthmatic patients who have not been treated with corticosteroids (7 25) Hypo-adrenalism may reduce the tolerance to status asthmaticus of corticosteroid treated asthma pa tients (6 24) The danger is greatest 2–3 weeks after the discontinuance of long term corticoste roid therapy (4) but it may take as long as a year in some cases before normal response of the adrenal cortex is restored in stress situations (16) Thirty of our 54 patients had been treated with corticosteroids before admission to hospital The adrenocortical insufficiency in the four cases in which no corticosteroids were administered in hospital may be regarded as the iatrogenic factor contributing to death

Respirator treatment of moribund asthmatics is in our experience a highly efficient life saving measure In another material (11) 26 patients were treated a total of 29 times for an average of five days with controlled ventilation the status asthmaticus ended fatally in only four of these cases Two of the fatalities were due to technical complications of the respirator therapy The earliest possible observation of the respiratory in sufficiency which requires careful supervision would perhaps save the life of many asthmatics

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PROLONGED ARTIFICIAL VENTILATION IN SEVERE STATUS ASTHMATICUS

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Abstract Prolonged artificial ventilation was employed 29 times in cases of severe status asthmaticus at the Medical Department, University of Turku in co-operation with the Department of Anesthesiology Three of the 26 patients were twice under respirator treatment. The outcome was fatal in four cases At the time they *were connected up to the respirator all the patients were* physically exhausted pale cyanosed and had difficulties in coughing up bronchial secretions Seventeen patients were unconscious. Carbon dioxide retention occurred in all the cases The mean duration of controlled mechanical ventilation was five days

The indications for controlled respirator therapy are examined with reference to the material The conclusion reached is that this form of therapy is useful, often a necessary and lifesaving measure in the treatment of patients with most severe status asthmaticus

Asthmatic patients who fail to respond clinically to drugs fluid therapy and other general treatment constitute a serious problem Recently in addition to aiding the patient s own respiratory function by intermittent positive pressure breathing (IPPB) a severe ventilatory insufficiency in asthmatic patients has been treated by using controlled ventilation transferring the ventilatory function as a whole to a machine Apart from the reports reviewed by Ambiavagar and Jones in their impressive paper (2) there have been few reports concerning this kind of treatment (3 6 7 16) Since 1962 respirator treatment of this kind based on controlled ventilation has been given at the Department of Medicine University of Turku in co-operation with the Department of Anesthesiology The material the method of respirator treatment and the results are described in this paper

MATERIAL

The annual number of asthmatic patients treated at the Medical Clinic, University of Turku and the number of patients treated with controlled ventilation are given in Fig 1 Three of the 26 patients were twice under respirator treatment making a total of 29 treatments The mean age of the patients was 48 years (range 24–76) There were 0 women and six men The onset of asthma occurred after the 40th year in eight cases There was *only one case in which the history of asthma was less* than two ye rs it was ‿–5 years in four patients 5–15 years in ten, and over 15 years in 11 cases Four of the asthmatic patients given respirator treatment were hospitalised for the first time for asthma 12 had been admitted 1–3 times and ten over four times Eight patients had received corticosteroids before admission The history of 11 patients displayed exogenous asthma provoking allergens and infection was the provoking factor in 14 cases The electrocardiography and chest roentgenogram findings are given in Table I

The time in hospital before the institution of respirator therapy varied from immediate resuscitation on admission to 23 days (mean 3 days) Signs of infection (elevated sedimentation rate leukocytosis sinusitis, pyrexia etc) were noted in 12 cases before the patient *was placed in the respirator*

Status at the Time when the Patient was Connected to the Respirator

The principal symptoms are listed in Table II All the *patients were physically exhausted pale cyanosed and* had difficulty in coughing up bronchial secretions Seventeen patients were unconscious. All the patients had carbon dioxide retention, but mild in five cases. The capillary blood Astrup values were determined in one of these five patients only after intubation and manual ventilation and in two cases several hours before the rapid exacerbation of dyspnea and cyanosis

Recovery with the aid of conventional clinical drug, fluid oxygen etc therapy appeared hopeless in all the cases given respirator treatment because the status asthmaticus was progressive or the situation deteriorated suddenly Several patients had in addition to lowered blood pressure also pronounced tachycardia (120–150/min) and symptoms of clinical shock. In two cases respirator therapy had to be resorted to on account of cardiac arrest and in three cases because of respiratory arrest

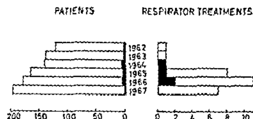


Fig 1 The annual number of patients hospitalised for asthma and of those treated with controlled ventilation The deaths are indicated in the columns in black

The Method of Respirator Treatment

To avoid the risk of aspiration a stomach tube was introduced and the patients were intubated using succinylcholine as a relaxant Those who were conscious restless and reacted to stimuli were then anesthetised either with halothane or in the first years with ether Manual ventilation with 100 oxygen was applied for 10–30 min after which the patients were connected up to an Engstrom respirator (in three cases to a Bennet respirator) Tracheotomy was performed in 22 cases intubation sufficed in seven cases Repeated bronchial lava es were performed with ca 10 ml of sterile saline solution especially in the earlier cases when there were no effective humidification methods Especially in the early days, lavage was often performed by bronchoscopy Tracheal suction was first carried out at 30–60 min intervals later as required Sixteen patients were kept nder ether or halothane anesthesia for the first 24 ours sometimes even longer Eighteen patients were given 10–15 mg of tubocurarine at 1–2 hour intervals in order to maintain muscular relaxation (an average of ca 400 mg in all per patient) Succinylcholine was given to two patients while sedatives sufficed in nine cases to adapt the patient to the respirator All patients received antibiotics and fluids intravenously The electrolyte and fluid balance were followed and corrected when necessary Oxygen and inhaled air were humidified in later years by an ultrasonic humidifier All the patients were also given hydrocortisone (usually 100–300 mg per day) and several received the customary bronchodilators

Table I *The ECG and chest roentgenogram findings of the asthmatic patients before or at the beginning of respirator treatment*

Some of the electrocardiograms were taken several days before the acute exacerbation of the disease

Electrocardiography	
Normal	6
P pulmonale	14
Right ventricular strain	9
Other abnormalities	2
Chest roentgenogram	
The lungs	
Normal	11
Emphysema or fibrosis	13
Other abnormalities	1
The heart	
Normal	19
Cor pulmonale	4

Table II *Condition of the asthmatic patients at the time they were placed in the respirator (29 cases)*

The pH and pCO_2 were recorded from capillary blood according to Astrup

Consciousness	
Conscious	7
Confused	5
Unconscious	17
Respiratory arrest	3
Cardiac arrest	2
Retention of CO_2 (pCO_2) mm Hg	
30–45	0
46–60	5
61–90	12
Over 90	9
No information	3
Acidosis–Ph	
7 45–7 35	0
7 34–7 20	10
7 19–7 05	14
Under 7 05	2
No information	3
Systolic blood pressure	
Over 160 mm Hg	1
Normal	6
Under 100 mm Hg	14
No information	8

Hypercapnia began to decrease in the majority of the cases immediately after starting manual ventilation and respirator treatment There was concurrent elevation of pH Seven patients were given alkalising therapy for severe acidosis In five of these cases 500 ml of trishydroxy methyl amino methane (THAM) was infused as 0 3 molar solution In the two cases of cardiac arrest concentrated sodium bicarbonate was given in the conventional way

The peak endotracheal inflation pressure was always distinctly elevated at the beginning of therapy ranging from 34 cm H O to above the upper limit of the scale (over 70 cm H O) in many cases Correction of hypercapnia normalisation of pH restoring of the electrolyte balance reduction of the endotracheal inflation pressure to normal or below 40 cm H O and improvement of the patient s own respiratory function and general condition were signs permitting the patient s removal from the respirator The treatment time in the respirator ranged from 22 hours to 14 days mean five days While the patient was being weaned from prolonged controlled ventilation the patient s own respiration was assisted intermittently by a Bennet respirator usually for 2–3 days

Failures in Respirator Treatment

Four of the 29 respirator treatments ended in the patient s death Two of the deaths were due

to technical complications Irreversible cerebral anoxia had obviously developed in the third case before the respirator treatment for the patient did not regain consciousness and his pupils re mained maximally dilated although airway re sistance and hypercapnia diminished The patient developed high fever in the terminal phase and autopsy r vealed in addition to abundant mucous plugging in the bronchi numerous small hemor rhages and emollitions in the brain In the fourth fatal case there was no decrease in hypercapnia (pCO 98 mm Hg) during 22 hours in the respirator cyanosis and hypotonia persisted At autopsy a large amorphous mass of mucus was found completely blocking the lumen of the large bronchi A woman aged 68 made a good recovery from hypercapnia and status asthmaticus but died two weeks after the respirator treatment from pulmonary embolus We considered that this pa tient recovered under respirator treatment.

DISCUSSION

In our opinion most of the 29 status asthmaticus cases would have terminated fatally without quick and active therapeutic measures The good therapeutic result achieved supports the justifica tion for controlled ventilation Seventeen of the patients were unconscious when the therapy was started We have no control series to corroborate our belief but the history and clinical findings of the patients resemble those of a group that died of asthma in the same clinic (8) The common ness of deaths from asthma and their clinical unexpectedness also warrant the search for ef fective countermeasures.

Lowered oxygen tension of arterial blood is common even in asthmatic patients who are in a moderately quiescent phase (19) This reduced oxygen tension renders the patient very vulnerable to further increased obstruction of the airways and dangerous levels of hypoxemia may develop rapidly when the asthma is exacerbated Broncho-dilating drugs although they reduce airway re sistance effectively do not diminish hypoxemia (14 15) and may even aggravate the situation by intensifying the ventilation perfusion disturbance in the lung (13) Overdosage of these drugs is a risk in itself (8) It thus appears very logical in severe status asthmaticus when hypoxemia is extremely pronounced to try to eliminate it by artificial ventilation

The good therapeutic results obtained at our clinic and elsewhere (2 6 7 12) in the manage ment of clinically hopeless cases argue for the efficiency of prolonged artificial ventilation in the treatment of status asthmaticus In only one of our cases did the hypercapnia remain uncor rected despite controlled ventilation for 22 hours It has been asserted that despite the increase in minute ventilation alveolar ventilation decreases and the blood gas situation may deteriorate in connection with severe obstruction (10) High inspiratory pressure has been suspected to cause bronchiolar collapse (11) and it has been reported that physiologic dead space increases (18) Rapid reduction of hypercapnia by mechanical aids may be accompanied by diffuse neurologic signs coma and death possibly due to intracellular alkalosis of the brain (5) Hypercapnia must therefore be corrected gradually avoiding radical changes

A matter of decisive significance is determina tion of the presence of indications for controlled ventilation It goes without saying that cardiac arrest and respiratory arrest indicate the institu tion of treatment Loss of consciousness on ac count of hypercapnia and hypoxia is also re garded fairly generally as indicating controlled ventilation but the decision is considerably more difficult to make in other cases Accumulation of secretions in the airways which the patient can not cough up acceleration of the heart rate and inability to co-operate in aerosol and IPPB therapy are reasons for seriously considering con trolled ventilation

The sudden deaths from asthma (4) and the clinical severity of prolonged status asthmaticus pose a difficult problem for the clinician Our results show that artificial ventilation can also have a fatal outcome and the point was stressed recently in a leading article in The Lancet (1) It has been emphasised that a patient with asthma of cyano ic type who has once suffered carbon dioxide retention is particularly prone to a return to this condition (9) Schilling (16) suggested controlled ventilation in all cases with an arterial pCO higher than 80 mm Hg Determination of the oxygen tension of arterial blood in serious conditions offers an earlier basis of evaluation than carbon dioxide retention (19) Possible other

criteria are the reduction in blood pressure which may be a direct consequence of the diminution of the stroke volume of the heart on account of hypoxemia (17) a sharply increased heart rate (2) start of confusion rapidly increasing cyanosis incipient right congestive heart failure and extreme physical exhaustion It is in fact ad visable to avoid unfounded use of controlled ven tilation and instead give IPPB correctly and suf ficiently early This would make it possible to avoid unnecessary intubation which may itself provoke an attack However we wish also to emphasize the importance of immediate resuscita tion and intubation when there is no time for detailed deliberation We consider that it is im portant to be able to have a machine completely take over the increased work of breathing that an exhausted asthmatic has to contend with

The technical performance of controlled venti lation varies and even in our series the procedure was not always consistent down to the last detail Tracheostomy is a widely debated point It has the advantages of reducing the dead space giving a better chance of removal of bronchial secretion and allowing longer term treatment (17) An addi onal advantage is that the patient can adapt easily to tracheostomy than to intubation he principal arguments against tracheostomy are increased risk of infection the danger of hemor rhage and scar formation which complicates its repetition Although tracheostomy was chosen distinctly more often in our series we cannot deny that intubation alone would have been suf ficient in some of the cases

Another controversial detail is the use of muscle relaxants during prolonged artificial ventilation Lung compliance is said to diminish when muscle relaxants are administered (11) Muscle relaxants do not improve alveolar ventilation and they increase the patient s distress (2) In our experi ence however it is often impossible to adapt the patient to the respirator with the aid of sedatives alone although an endeavour should be made to achieve adaptation by means of manual ventila tion analgesics and sedatives without relaxing the patient

Acidosis reduces the bronchodilating effect of sympathomimetics Tracheostomy and artificial ventilation do not have an immediately correcting effect on deep acidosis and hypercapnia If the drugs used for bronchoconstriction are ineffective the effect of ventilation may remain insufficient Therefore alkalising drugs have been recom mended for severe acidosis (6 7 12 17) Intra venous sodium bicarbonate is commonly used However Holmdahl et al (6) prefer THAM (tris hydroxy methylamino methane) because aci dosis in status asthmaticus is due to deficient carbon dioxide elimination Alkalising therapy was administered to only seven of our cases, THAM was used in five and Na HCO_3 in two cases of cardiac arrest We are of the opinion that THAM quickens the relaxation of broncho constriction However as other drugs were used in liberal quantities at the same time this favor able effect cannot be proved convincingly

Important points to remember in connection with the use of a respirator are sufficient humi dification of the inhaled air careful aseptic treat ment of the airways the patient s general treat ment and early mobilisation

Prolonged artificial ventilation of moribund asthmatics proved to be one of the most favor able groups prognostically in an analysis (20) of respirator treatments (260 cases) at our hospital

The results achieved show the efficiency of the therapeutic method especially as it seems highly probable that the patients in question would have been lost without respirator treat ment The method is worth developing Finally co operation between different teams of physi cians is important and the treatment should be centralised Good training of the nursing staff and the immediate readiness for use of the ap paratus help to reduce the possibility of compli cations associated with the application of con trolled ventilation

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REITER S SYNDROME A FOLLOW UP STUDY

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Abstract During the last war there was an epidemic of dysentery in Finland on the Karelian Isthmus Out of a total of about 150 000 cases Reiter s syndrome developed in 350 (0.2) A follow up study of 100 of these cases was made about twenty years later The most frequent late changes were rheumatoid spondylitis (32%) chronic arthritis (18) and iritis (7) Occasional arthralgia without objective symptoms was also common

After termination of the first stage 20 patients (20) had become entirely asymptomatic The disease resulted in permanent disability in 42 cases (42) The importance of prostatitis as a focus of infection in this disease seems open to doubt The control study was based on 100 men examination of the prostate and roentgenography of the sacro-iliac joints and lumbar spine were performed

Reiter s syndrome has been regarded in many quarters as a disease which heals spontaneously without giving rise to residual changes (6 15) Mainly on the basis of recent studies this disease is now considered however to comparatively often become chronic or recur (4 7 13 17 26 29) On the other hand several French investigators reporting on fairly extensive series from the period of the Algerian war have stressed that so far scarcely anything can be stated about the long term prognosis of this syndrome (21 25)

MATERIAL AND METHODS

In Finland on the Karelian Isthmus there was an epidemic of dysentery during the last war with a peak occurring in the summer of 1944 In a total of about 150 000 dysentery cases Reiter s syndrome developed in 350 i.e in 0.2 Their treatment was concentrated in one war hospital In 1948 Paronen published an extensive investigation (20) based on 344 cases of Reiter s disease treated at the hospital mentioned A follow up study on 100 of these cases was made during the period 1963–67 i.e about twenty years later

A questionnaire was sent to all the subjects studied by Paronen and living in various parts of Finland requesting them to complete the form It appeared that 75 of the patients had either died gone abroad or their whereabouts was unknown For some other reason 117 patients failed to answer the inquiry and thus the final total of responses was 152 Of these 100 complied with the request to attend for personal examination by us This examination took place at the Outpatient departments of the Central Hospitals of the different areas. The data received earlier were supplemented by the attending physicians and hospitals where the patients had been treated It may be added that of the 52 subjects who replied but did not present themselves for examination 22 stated that they had had practically no symptoms since the first stage the others had symptoms mainly in the joints Particular attention was paid to the following points

A Clinical examination including prostate studies using the criteria presented by Romanus (23 24)

B Laboratory studies (sedimentation rate (Westergren) Waaler Rose urine studies electrocardiogram) Prostatic secretion was studied in 24 cases Five patients were examined by phonocardiography

C Roentgenologic studies (chest lumbar spine and sacro iliac joints) In addition roentgen films were made of the thoracic spine in 57 cases the cervical spine in 11 cases and the joints of the extremities in 48 cases according to the presence of symptoms The films were interpreted independently by a rheumatologist and a roentgenologist There were very few differences in interpretation and these were dealt with in consultation

Osteoporosis was not taken into account as a separate criterion In the case of sacro-iliitis sclerosis together with erosion of the joint space and ankylosis were considered positive findings Films of the sacro-iliac joints were taken in antero-posterior or antero-posterior oblique projection and films of the spine anteriorly and laterally

D Certain supplementary examinations Some patients were admitted to our hospital for further studies Arthrotomy of the knee joint was performed and the specimens obtained were examined histologically in five cases.

E Working capacity The cases were divided into four groups Grade I Fully employable Grade II Light or part time work Periodically incapable of working Grade III Employable Can do light housework Grade IV Confined to house but able to care for themselves

Table I *Roentgenologic spinal changes resembling rheumatoid spondylitis*

Changes	No of cases
Unilateral sacro iliitis	1
Symmetrical sacro iliitis	12
Symmetrical ankylosis of sacro iliac joint	4
Symmetrical sacro iliitis + squaring syndesmophytes	8
Symmetrical sacro iliitis + bamboo spine	2
Symmetrical ankylosis of sacro iliac joint + bamboo spine	5
Total	32

The series consisted of 93 men and seven women Age at onset of illness varied from 21 to 41 years with an average of 29 years at the time of the follow up the average age was 50 years During the first stage in 1944 the disease had been manifested as a triad in 79 cases whereas it had been disymptomatic in 15 and mono symptomatic in six cases Myocarditis was noted at this stage in six patients and pericarditis in one thus a total of seven patients had heart lesions

The control series consisted of 100 unselected male patients treated in the Medical Department their age distribution was consistent with that of the Reiter pa tients The prostate was examined Roentgen films of the sacro iliac joints and the lumbar spine were included

RESULTS

Twenty patients were completely cured after ter mination of the first stage

On the basis of spinal findings and peripheral joint symptoms the cases could be divided into different groups

Cases in which there were in addition to other symptoms roentgenologic changes similar to those in rheumatoid spondylitis (32 cases)

Cases with long standing joint symptoms but no changes pointing to rheumatoid spondylitis (18 cases)

Cases with joint symptoms which had either disappeared by 1947 or were slight and tem porary (30 cases)

Cases Similar to Rheumatoid Spondylitis

Roentgenologic examination showed spinal changes in this group (Table I) It should be noted that the spinal changes were limited to the areas of the lumbar spine and the lower part of the thoracic spine In one case there were also changes in the cervical spine Syndesmophytes occurred in a fairly localized area Fifteen sub jects showed also degenerative changes in the spine

On physical examination 13 patients were found to have comparatively mild spinal symp toms The others stated that they had motion tenderness back pain even at rest and stiffness Definite limitation of motion occurred in 14 cases a marked degree of thoracic kyphosis in only three Taking into account the considerable degenerative changes the spinal symptoms were relatively mild compared to the symptoms pres ent in rheumatoid spondylitis

Nineteen patients presented symptoms or changes in the joints of the extremities (peri articular swelling motion tenderness pain or limitation of motion) These symptoms most fre quently affected the lower extremities the hip knee and ankle joints and the proximal joints of the toes These changes were usually monoartic ular Hammer toes had developed in three cases

Roentgenologically there were joint changes in ten patients and calcaneal spur in nine (Table II) The sedimentation rate was increased (15–

Table II *Roentgenologic findings in the extremities in cases resembling rheumatoid spondylitis*

	Roentgenologic findings				
Changes in	Periosteal changes	Narrowing of the joint	Erosion	Subluxation	Ankylosis
Proximal interphal joint		1	1		
Hip joint			1		1
Knee joint	1	2	2		
Talocrural joint	1				
Metatarsophal joint		2	2	3	
Calcaneus	9				

81 mean 35) in fourteen patients The Waaler Rose test was negative in all the cases

About half the patients in this group had had lumbal pain at the first stage

Cases with Long standing Joint Symptoms but Without Changes Indicative of Rheumatoid Spondylitis

Physical examination showed joint changes in 18 patients *the knee finger and toe joints being* most often affected These changes were usually mono- or diarticular Periarticular swelling was the most common Knee hydrops had recurred in one patient two or three times a year Practically all had pain in addition to motion tenderness Morning stiffness did not occur in this group The articular symptoms had been continuous in only one case mostly they were periodical recurring at intervals ranging from one week to four months and lasting from one week to several months every year In autumn and in wet weather generally the symptoms were clearly exacerbated exertion had a similar effect In five cases the pain could be termed joint attack because of its acute nature In these latter cases the other symptoms of the triad were usually lacking Occasionally joint symptoms could be so severe that crutches were necessary

Roentgenographic involvement was extremely infrequent in this group Erosion was present in only two patients and calcaneal spur in two

The sedimentation rate was increased at the time of the examination in four cases and the Waaler Rose test was positive in one case

In the above cases in which long standing joint symptoms dominated the clinical picture differed to a great extent from classical rheumatoid arthritis It seems that rheumatoid arthritis does not develop on the basis of Reiter s syndrome (Table III)

Joint symptoms had been prominent for decades in many of the case histories

Case report (case 48)

Patient, male onset of Reiter s syndrome when aged 26 e g right ankle *affected during first stage in 1944 This* ankle had continued to be tender with occasional swelling Since 1946 both knees swollen at times In 1946 1955 and 1959 the symptoms were very severe patient was incapable of manual work for months After 1959 his condition improved slowly In 1964 only slight motion tenderness was demonstrable in the right ankle Roentgenologically there was evidence of dorsal and plantar calcaneal spur and of erosion in the talus

A joint which was affected at the late period of Reiter s syndrome was often though not invariably attacked also at the first stage

Other Cases with Joint Symptoms

Occasional arthralgia in the absence of objective findings was not infrequent It is noteworthy that following the first attack and a subsequent almost completely asymptomatic period over 20 years could elapse before a fresh attack occurred

Case report (case 100)

Patient male Reiter s syndrome developed in 1944 when he was aged 28 Among other joints left ankle involved Patient had been asymptomatic except for occasional tenderness of this ankle after exertion In spring 1966 suffered from diarrhea probably of viral origin In the lat stages of the disease the typical triad was observed with increase in temperature and sedimentation rate Left ankle became swollen and so very painful that patient had to use crutches for some weeks The symptoms disappeared in about a month

Some other changes which appeared in this material will be discussed in the following

Heart lesions

There were seven patients with cardiac involvement during the first stage Five of these were

Table III *Comparison of findings in cases of long standing joint symptoms with certain criteria of the American Rheumatism Association*

Criteria of rheumatoid arthritis	Reiter s syndrome
1 Morning stiffness	–
2 Pain on motion or tenderness in at least one joint	+
3 Swell ng in at least one joint continuously for not less than six weeks	±
4 Swelling of at least one other joint	±
5 Symmetrical joint swelling	–
6 Subcutaneous nodules	–
7 Roentgenological changes typical of rheumatoid arthritis	±
8 Positive Waaler–Rose test	±
9 Poor mucin precip tate from synovial flu d	+
10 Characteristic histologi changes in synovial membrane	+
11 Characteristic histolog c changes in nodules	–

Classical rheumato d arthritis is to be diagnosed only if seven of the eleven criteria are present

Table IV *Incidence of iritis*

Case no	Frequency in 1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964
31									+	+								+	
32										+	+	+	+	+					
34											+	+			+				
42																	+		
57					+			+											
58														+		+		+	
72	+	+						+											

practically asymptomatic at the follow up cardiac insufficiency had developed in one patient and disturbance of heart rhythm total heart block in another A mitral vitium had appeared in one case but this could not be correlated with Reiter s syndrome because rheumatic fever had occurred earlier

Ocular changes

Conjunctival irritation (photophobia pain and redness) occurred occasionally in nineteen subjects usually bilaterally This lasted about a week or two in some cases it occurred once a year in other only a few times at intervals of several years Usually medication was not required This eye symptom can probably not be definitely ascribed to Reiter s syndrome

Iritis usually unilateral had appeared in a total of seven cases (Table IV) There was recurrent iritis in all patients except one In one case the disease resulted in secondary glaucoma and considerable loss of vision (case 72)

Case report (case 72)

Patient male at the first stage presented complete triad subsequently several joints involved almost uninterruptedly Recurrent iritis since 1946 hospitalized for this also treated in outpatient departments Bilateral uveitis and secondary glaucoma diagnosed in 1953 Bilateral iridectomy performed In 1966 vision in right eye was 0 67 in left 0 17

The eye symptoms notably iritis did not seem to be related to the joint attacks but to occur independently In four cases there were changes resembling rheumatoid spondylitis

Cutaneous changes

Two patients had mild psoriasis but not peripheral joint symptoms Two other patients showed cutaneous changes a dry scaling eczema which was stated to have been similar in the early stage In one of the patients these changes had been present continuously in the other they had recurred in 1957

Gastrointestinal symptoms

There were 22 persons who had had symptoms of diarrhea for many years without any detectable cause Among these 11 were symptom free in other respects Exacerbation of diarrhea caused accentuation of joint symptoms in two cases

Lesions of the central nervous system

Parkinson s disease developed in one subject who had had encephalitis at the first stage of Reiter s syndrome The patient was completely incapacitated in a fairly short time

Case report (case 16)

Patient male aged 38 developed Reiter s syndrome (triad) in 1944 Since onset severe headaches aggravated e g by head movements The headaches previously had not suffered from this complaint lasted for about two months and were interpreted as due to encephalitis Except for symptoms of arthralgia well until 1956 when parkinsonism developed completely incapacitating him in a couple of years Greasy skin and excessive salivation were suggestive of encephalitis

Urogenital symptoms

None of the subjects except one had symptoms of urethritis after 1947 Urine studies showed aseptic pyuria in eight subjects two of whom had also prostatitis The incidence of prostatitis in the Reiter patients and controls is illustrated in Table V

The finding was much more often positive on palpation than in secretion tests If only the palpation finding is taken into account 24 % of the

Reiter patients had prostatitis against 31° in the control group The group of patients whose condition resembled rheumatoid spondylitis included nine in whom prostatitis developed

Women affected with Reiter's syndrome

Paronen s series included 34 women (10°) As stated in the foregoing, seven of these attended th follow up In spite of the inclusion of these patients in the groups dealt with above they seem to merit consideration here Three women reported they had been completely cured after the first stage Two had had symptoms of arthralgic or of an arthritic type in the toe and finger joints and one knee hydrops which recurred repeatedly Iritis had recurred in one woman Ro ntgenologic findings were normal in five instances Calcaneal spur was found in two women one of them showing also ankylosis of the sacro-iliac joints This last change was silent i e asymptomatic There were no cases of inflammation of the genital tract

Changes in the knee joints

Crepitation appeared in the patella in several of the subjects who had had knee hydrops during the first stage This crepitation was also present at the follow up Arthrotomies of the knee joint were therefore performed and the subjects were found to have a considerable degree of chondromalacia

Working capacity

The patients were classified according to working capacity in four grades as mentioned above (Table VI) The disease had caused more or less severe disability in 42 % of the patients Three patients had been completely incapacited

Table V *Prostatitis in Reiter patients compared with control group*

	No cases	Palpation finding + (cases)	Secretion examined (cases)	Secretion + (cases)[a]
Reiter s syndrome	93	2_	24	8
Control group	100	31	40	16

Over 10 leucocytes per high power field

Table VI *Working capacity of Reiter patients rated on the basis of follow up*

Grade	No of cases
I	58
II	39
III	2
IV	1
Total	58 + 42

Since duration of the first stage of Reiter s syndrome may probably be regarded in the nature of a criterion of severity the patients were compared in respect of this duration and reduced working capacity The severity of the disease did not seem to be directly related to long term prognosis

Roentgenologic changes in control series

Two control subjects showed changes similar to rheumatoid spondylitis Degenerative changes in the lumbar spine were noted in 85

DISCUSSION

From the series studied it seems that Reiter s syndrome was a disabling disease in about half of all the cases or at least caused symptoms from which the patients suffered for decades Evaluation of the extent to which the patients are disabled by Reiter s disease—a question of great economic importance to war veterans—presents certain difficulties First the cases must be cleared as far as possible of any changes arising from other causes such as arthroses Opinions on arthrosis however seem to be changing The term modele arthrosique has been introduced as well as arthrotic reaction (11)

It appears as if Reiter s syndrome could produce changes of an arthrotic type mainly chondromalacia Nonetheless it is not possible while studies are still in progress to draw definitive conclusions The periodical occurrence of symptoms sometimes at long intervals and the fact that uninterrupted observation of cases has not been possible make evaluation difficult

When the disease had become protracted or recurred only one of the cardinal symptoms was

usually present There could however be a recurrence of the complete triad as long as 20 years after the first stage

About ten cases of aortic insufficiency attributed to Reiter s syndrome have been reported up to the present (5 22) It does not seem however that all of these cases can be definitely considered due to this syndrome This has moreover been pointed out elsewhere (28) Insufficiency of the aortic valve was not diagnosed in the present series of patients It has been reported that while Shigella infections may cause permanent heart lesions these occur only rarely if at all in cases due to Flexner s bacillus (27) The patients here studied had had Flexner s dysentery

Comparatively few neurologic symptoms have been described in association with Reiter s syndrome nevertheless cases of neuritis and encephalitis have occurred (17)

There has been difference of opinion whether rheumatoid arthritis can develop on the basis of Reiter s syndrome Rheumatoid arthritis caused by this syndrome was not found in the present material—an observation which accords with Good s (7) results Though it has been stated that what are known as root joints are not affected (7) a lesion of the hip joint for example did occur among the patients reported on here

The incidence of sacro-iliitis was relatively high as stated earlier (16) These changes were usually bilateral Apparently the disease progresses rather slowly in the spine and may come to a standstill so that it remains restricted to the sacro iliac joints If changes occur in other areas of the spine they are rather localized In fact it has been stressed that isolated bridge formations are typical (7) Calcaneal spur seems also to be fairly common (7 14)

The symptoms caused by spinal changes and calcaneal spur were rather mild and did not greatly disturb the patients in this series Rheumatoid spondylitis associated with Reiter s syndrome closely resembles the type occurring in women Its symptoms as is well known are often much milder than in men and moreover peripheral joint lesions particularly of the toe and finger joints are fairly common (9 10)

Reiter s syndrome seems to be associated with chronic arthritis or arthralgia in which articular symptoms as it were fluctuate sometimes being slight or even absent whereas at other times they are very severe the roentgenologic findings however are usually negative

As previously pointed out Paronen s (20) study included a considerable number of women Since the patients in this series were predominantly from the theatre of operations where there were few women the impression is gained that Reiter s syndrome affects women comparatively often

The part played by chronic prostatitis or prostato vesiculitis in rheumatoid spondylitis and Reiter s syndrome has been emphasized especially in Sweden (18 24) Olhagen (18) reported an incidence of prostatitis exceeding 80% Prostatitis has been considered a focus of infection important in the development of this syndrome In the present study the incidence of prostatitis was low there were even cures in the course of years On the other hand some authors (12 19) studying extensive case materials found that a normal series may include about 30% with prostatitis It has been reported on the basis of autopsy findings and needle biopsy specimens, that in what is known as chronic prostatitis it is only very rarely a question of inflammation (1) There seems to be congestion rather than infection in most patients Nor do the results of lymphography definitely support the role of prostatitis in cases of rheumatoid spondylitis (3) Attempts to treat prostatitis have failed to disclose definite evidence as regards the basic disease

Thus the importance of chronic prostatitis as a focus of infection in Reiter s syndrome seems for the present, to be questionable On the other hand some experimental studies have shown that antibodies against prostatic tissue occur in Reiter s syndrome (8)

It has been claimed that ankylosis of the sacro-iliac joints begins to appear in men over 35 years and is fairly frequent in men over 50 (2) This study failed to confirm this opinion in the control material

The extent to which this series is representative should also be dealt with briefly Since attendance was voluntary it was probably exactly those patients who felt they were cured that failed to present themselves for examination Consequently the series may include a relatively greater proportion of patients suffering from aftereffects of Reiter s syndrome

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ANTICOAGULANT TREATMENT OF ACUTE CORONARY THROMBOSIS

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Abstract Four hundred and fifty-one patients with acute myocardial infarction were placed according to their date of admission in one of two groups an anticoagulant treated group and a placebo group After the exclusion of patients who died within 48 hours patients whose diagnosis was only made post mortem, and a few patients who for various other reasons had to be left untreated there remained 276 patients for the trial 156 patients in the anticoagulant treated group and 1 0 patients in the placebo group The results in terms of mortality rate thrombo-embolic complications and haemorrhagic incidents were evaluated after 4 weeks treatment (short term material) and again after an observation period of up to two years in the 139 anticoagulant treated patients and the 95 placebo patients who survived the first four weeks (long term material)

In the short term material the anticoagulant treated group showed a statistically significant reduction in the number of thrombo-embolic complications and the mortality rate In the long term material there was a statistically significant reduction in the mortality rate in anticoagulant treated patients who during the acute stage had been classified as bad risks and in the recurrence rate for patients over 60 In only one patient during long term treatment, could death be ascribed for certain to this treatment

During the last 15-20 years many trials with anticoagulant treatment of myocardial infarction have been published but agreement has not been reached on the value of this form of treatment

The question has ususally been considered from one of two aspects 1 what is the effect of anticoagulant treatment in the acute stage of myocardial infarction?—i e the 4-6 weeks following infarction and 2 what is the effect of long term treatment? here observation periods range from one to 15 years

We present here the results of a controlled trial of anticoagulant treatment of myocardial infarction where treatment was begun in the acute phase of the disease and continued for up to 24 months average 17 months

MATERIAL AND METHODS

The material comprises 451 patients with acute myocardial infarction admitted to medical department E of Frederiksberg Hospital during the period from January 1 1961 to December 31 1964 The study was concluded on July 1 1966 the maximum observation period was two years Infarctions more than two weeks old on admission were not included

Acute myocardial infarction was diagnosed when two or more of the following criteria were met

1 typical history and clinical picture
2 typical ECG changes
3 elevated levels of serum glutamic-oxalo-acetic transaminase

General treatment consisted in strict bed rest for at least 3 weeks followed by gradual mobilization Uncomplicated cases were discharged after 4-5 weeks Cardiac arrhythmias and decompensation were treated with digitalis along conventional lines, while diuretics were used sparingly Shock was treated with metaraminol and pain usually with meperidine

Patients admitted on odd days were treated with heparin and dicoumarol while those admitted on even days had placebo treatment The dose of dicoumarol was 300 360 mg during the first 24 h followed by 60 mg the next morning The prothrombin level was taken as a guide for subsequent dosage Heparin 25 000 i u was given subcutaneously twice daily until the prothrombin level was below 30% Prothrombin was determined by Owren s method values between 10 and 30 being aimed at

Patients in the placebo group were given 1 ml of 0 9% sodium chloride subcutaneously twice daily for two days and tablets containing lactose and starch

Prothrombin values were checked in both anticoagulant treated patients and placebo patients at intervals of 1-3 days during admission and every second to fourth week after discharge

The effect of treatment has been analyzed separately for the acute stage and the subsequent period the boundary being set at one month The composition of the material is shown in Table I The difference between the number of patients admitted on odd and on even days is not significant The table shows that a number of patients were excluded from further study 1 patients with preexisting disease that contraindicated anticoagulant

Table I *Composition of the material*

	Odd days	Even days	Total
Total no admitted	244	207	451
Died within 48 h	47	41	88
Diagnosed post mortem	17	26	43
Survived not treated or treated for less than one month	11	10	21
Died from 48 h to 30 d after admission untreated or treated for less than 48 h	13	6	19
Four patients transferred from placebo to anticoagulant treatment	0	4	4
Short term group	156	1.0	276
Deaths in short term group	17	25	42
Long term group	139	95	234

treatment 2 some patients whose mental condition pre cluded cooperation and 3 patients so ill that drugs could not be given by mouth Four patients were also excluded who started on placebo but later received anti coagulant treatment because of severe thrombo embolism all these four patients died

The remaining 276 patients constitute the short term material The number of patients admitted on odd and on even days 1°6 and 1°0 respectively do not differ significantly when compared with the total number of patients admitted on odd and on even days

The long term material comprises all the survivors in e short term groups These have been followed up for up to two years Here the difference between the num ber admitted on odd and on even days is significant in comparison with the total number admitted on odd and on even days

Both materials have been analyzed in terms of mor tality recurrence rate thrombo embolic complications and haemorrhagic phenomena

RESULTS

Short term group

The anticoagulant treated group and the placebo group are fully comparable Age and sex distribu tions are shown in Table II In addition the two groups were analyzed for comparability with respect to a number of properties (15 in all) that have a bearing on prognosis such as obesity diabetes hypercholesterolaemia hypertension heart size one or more previous infarctions pre vious angina pectoris heart failure or arrhythm mias type of ECG-changes the occurrence of shock heart failure or arrhythmias following an acute attack No significant differences were found though there were rather more patients with previous infarctions in the placebo group

Intensity of anticoagulant treatment

The effect of heparin administration on coagula bility was not studied The intensity of dicou marol treatment was evaluated by electronic data processing of all prothrombin values for each pa tient separately When a prothrombin level of 30% was taken as the upper limit of therapeutic effectiveness the patients were classified as fol lows

1 well controlled (PP values <30% for 70-100% of the time) 120 patients = 77 0 %
2 moderately well controlled (PP values <30% for 40-70% of the time) 30 patients = 19 2%
3 poorly controlled (PP values <30% for 0-40% of the time) 6 patients = 3 8 %

Mortality rate and thrombo embolic complications

In the entire material the mortality rate in the first month was 37 7% and for the whole short term material 15 2% (42 out of 276 patients)

Table III shows the mortality rates and causes of death in the anticoagulant treated and the placebo group The difference between the mor tality rates of the two groups is significant (0 05 > p > 0 02) As mentioned the placebo group showed an excess (not statistically signifi cant) of patients with previous myocardial infarc tion After exclusion of this category of patients the mortality rates were

14 deaths among 135 anticoagulant treated cases = 10 4

21 deaths among 95 placebo treated cases = 22 0%

Table II *Sex and age distribution in the short term groups*

Age at beginning of treatment (y)	Anticoagulant treatment (156)		Placebo treatment (120)	
	No	Per cent	No	Per cent
Male	112	71 8	79	65 8
Female	44	28 2	41	34 2
30–39	2	1 3	0	0
40–49	10	6 4	6	5 0
50–59	37	23 7	32	26 7
60–69	60	38 4	44	36 6
70–79	36	23 1	29	24 2
80–89	11	7 1	9	7 5
Mean age	64 5 y		64 6 y	

The difference remains significant (0 02 > p > 0 01)

Excluding patients where treatment was not started until 72 hours or more after the infarction we get

13 deaths among 111 anticoagulant treated cases = 11 7 %

20 deaths among 86 placebo treated cases = 23 3 %

Here also the difference is significant (0 05 > p > 0 02)

Table IV relates the mortality rate to age sex and the severity of the disease in the acute stage as judged by Russek s (16) criteria for good and bad risk patients

Thus we find that the mortality rate is significantly higher in the placebo group The apparently higher mortality rate among women is not significant (0 1 > p > 0 05) Nor is the difference in mortality rate between anticoagulant treated patients and placebo-treated men and women and between patients over 60 and those under 60 when these groups are considered separately No definite conclusions can be drawn from a comparison between mortality rates in good risk and bad risk patients

Table V shows the number of thrombo-embolic episodes (including recurrence of myocardial infarction) in the short term material The difference between the anticoagulant treated and the placebo-treated cases is significant (0 01 > p > 0 001)

When we exclude patients who died of thrombo-embolic complications (including reinfarction) and consider mortality rates we get

Table III Deaths in the short term groups

Cause of death	Anti coagulant treated	Placebo treated
Myocardial infarction which led to admission	16	18
Recurrence of infarction during admission	1	
Pulmonary embolism		1
Cerebral thrombosis + myocardial infarction		4
Embolism of aorta and renal artery + myocardial infarction		1
Multiple infarctions + myocardial infarction		1
Total deaths	17 = 10 9	25 = 20 8

Table IV *Mortality rates in short term groups related to sex age and good/poor risk category*

	Anticoagulant treated	Placebo treated
Male	10 of 112 = 8 9	13 of 79 = 16 5
Female	7 of 44 = 15 9	12 of 41 = 29 3
Age < 60 y	2 of 49 = 4 1	4 of 38 = 10 5
Age > 60 y	15 of 107 = 14 0	21 of 82 = 25 6
Good risks	1 of 44 = 2 3	4 of 31 = 12 9
Bad risks	16 of 112 = 14 3	21 of 89 = 23 6

16 deaths among 155 anticoagulant treated cases = 10 3 %

18 deaths among 113 placebo treated cases = 15 9 %

This difference is not significant (p > 0 1)

When we consider the patients with diabetes mellitus and/or obesity the difference in mortality rates between anticoagulant treated and placebo-treated patients is particularly marked

4 deaths among 43 anticoagulant treated patients = 9 3

15 deaths among 39 placebo treated patients = 38 5 %

Among the 43 anticoagulant treated patients in this category none died of thrombo-embolic complications (other than recurrence of myocardial infarction) while among the 39 placebo-treated patients six died of thrombo-embolism

A similar observation might perhaps have been expected in respect of patients with cardiac decompensation in whom immobilization was particularly severe and prolonged However the

Table V *Thrombo-embolic episodes in the short term groups*

Figures in brackets indicate deaths

	Anti coagulant treated	Placebo-treated
Recurrence of myocardial infarction	1 (1)	1 (0)
Cerebral thrombosis	2 (0)	6 (4)
Pulmonary embolism	0	2 (1)
Thrombophlebitis	1 (0)	1 (0)
Embolism of aorta and renal artery	0	1 (1)
Multiple emboli to internal organs and extremities		1 (1)
Total	4 = 2 6	12 = 10 0

Table VI *Sex and age distribution in the long term material*

Age at beginning of treatment (y)		Anticoagulant treated (139)		Placebo treated (95)	
		No	Per cent	No	Per cent
	Male	102	73 4	66	69 5
	Female	37	26 6	29	30 5
30–39		2	1 4	0	0
40–49		10	7 2	5	5 3
50–59		35	25 2	29	30 5
60–69		53	38 1	35	36 8
70–79		31	22 3	21	22 1
80–89		8	5 8	5	5 3

mortality rates for these patients were 31 3% (15 out of 48) for those who received anticoagulant treatment and 40 0% (18 out of 45) for placebo treated patients This difference is smaller than that found for the entire short term material

If we consider the short term material after exclusion of patients with diabetes and/or obesity the mortality rates for the anticoagulant treated and the placebo-treated patients are practically identical viz. 11 5% (13 out of 113) and 12 3% (10 out of 81) respectively

Haemorrhagic complications

All cases of internal haemorrhage and those requiring transfusion or where the haemoglobin concentration fell by more than 1 g/100 ml were regarded as severe bleeding

Among the anticoagulant treated patients 7 7% had haemorrhagic episodes during the first month Three patients (1 7%) had severe bleeding and ten (6 0%) mild bleeding In the placebo group one patient (0 8%) had mild bleeding In two anticoagulant treated patients death was associated with bleeding

One patient had severe haematemesis a few hours after administration of the initial dose of dicoumarol (300 mg) and heparin Phytomenadion 10 mg intravenously was given at once On the second day the prothrombin value was 60% on the third day 100% After another haematemesis on the second day bleeding ceased Six days later the patient died of the myocardial infarction Autopsy showed erosion in the cardia.

The other patient s condition was poor throughout after six days he went into shock and died Immediately before death there was a haematemesis of about 1/2 l the prothrombin value was then 15% At autopsy nothing was found to explain the bleeding

Long term groups

These comprise 139 anticoagulant treated patients followed on an average for 520 days, and 95 placebo-treated patients followed on an average for 522 days

A number of patients did not complete the full period of the study because they were unable or unwilling to cooperate 26 patients (18 7%) from the anticoagulant treated group and 20 patients (21 1%) from the placebo group The remainder were followed up for two years or until death

Table VI shows the sex and age distribution of the two groups As mentioned above the number of patients in the two groups differs significantly from each other as compared with the total figures for patients admitted on odd and on even days ($0.05 > p > 0.02$) Apart from this the two groups are fully comparable in respect of all properties studied ($p > 0.05$) In particular the difference with respect to previous myocardial infarction is not significant ($0.1 > p > 0.05$)

Intensity of anticoagulant treatment

Using the same criteria as for the short term material we find

1 well controlled 103 patients = 74 1%
2 moderately well controlled 34 patients = 24 5%
3 poorly controlled two patients = 1 4%

Mortality rate and thrombo-embolic complications

The number of deaths and their causes in the long term groups are listed in Table VII It is seen that death due to the cardiac disease thrombo-embolism or haemorrhage occurred in 13 out of 137 anticoagulant treated patients i e 9 5 and in 18 out of 94 placebo-treated patients i e 19 1% The difference is not significant ($0.1 > p > 0.05$) Table VII also shows that the deaths were evenly distributed over the observation period of two years

Two patients of the anticoagulant treated group died of intercurrent disease one died of pneu

Table VII *Deaths in the long term groups*

Cause of death	Anticoagulant treated Mo 1–2	Mo 2–6	Mo 6–12	Mo 12–24	Total	Placebo treated Mo 1–2	Mo 2–6	Mo 6–12	Mo 12–24	Total
Myocardial infarct on which led to *first admission*	3	1			4	2				2
First recurrence (+autopsy)				1	1	1	1	2	2	6
Second recurrence (+autopsy)				1	1					0
Sudden death (−autopsy)		2		1	3	1	2	2	4	9
Cardiac decompensation			1	1	2		1			1
Pulmonary embolism	1				1					
Haemorrhage			1		1					
Total	4	3	2	4	13	4	4	4	6	18
Intercurrent diseases	1			1	2				1	1

monia in another hospital The other was admitted with myocardial infarction and left sided hemiplegia. Death occurred 38 days after admission Autopsy showed scattered bronchopneumonia infarction of the right hemisphere and healed myocardial infarction The one death of intercurrent disease in the placebo group was due to carcinoma of the lung

After exclusion of patients with previous myocardial infarction there were 121 patients in the anticoagulant treated group One patient died of intercurrent disease Among the remaining 120 patients there were ten deaths i e 8 3% Similarly in the placebo-treated group there remained 73 patients with 13 deaths i e 17 8 This difference is not significant ($p > 0\ 1$) The ratio between the mortality rates remains unchanged

Table VIII shows the mortality rate in relation to sex age groups and whether in the acute stage the patients were assigned to the good or the bad risk group While there is no significant difference between the anticoagulant treated and the placebo-treated men or women or between patients over 60 and those under 60 there is a significant difference in favour of bad risk patients ($0\ 05 > p > 0\ 02$) in contrast to the findings for the short term mortality rate

The higher mortality rates for obese and/or diabetic patients in the short term group cannot be demonstrated in the long term material Among 39 anticoagulant treated patients in this category there were five deaths i e 12 8% and among 24 placebo-treated patients there were five deaths i e 20 8% This does not differ from the allover mortality rates of the two groups

Table IX shows the thrombo-embolic complications Recurrence of myocardial infarction includes 1 cases verified at autopsy 2 cases of sudden death according to the death certificates these were all due to myocardial infarction 3 nonfatal recurrences clinically verified during hospitalization

The difference between nine recurrences among 139 anticoagulant treated patients and 19 recurrences among 95 placebo treated patients is highly significant ($p < 0\ 001$) When male patients are considered separately the difference remains significant (6 9 recurrences in anticoagulant treated 19 7% in placebo treated men) The figures for women alone are too small to permit statistical evaluation

When the material is divided according to age there is a significant difference regarding patients over 60 (4 4% recurrences in the anticoagulant treated patients and 26 2% recurrences in the placebo-treated) whereas no difference is seen in patients under 60 (10 6% vs 8 8%) The recur

Table VIII *Mortality rates in long term groups related to sex age and good/bad risk category*

	Anticoagulant treated %	Placebo-treated %
Male	11 of 100 = 11.0	13 of 65 = 20 0
Female	2 of 37 = 5 4	5 of 29 = 17 3
Age < 60 y	3 of 47 = 6 4	4 of 33 = 12.1
Age > 60 y	[illegible]0 of 90 11 1	14 of 61 = 23 0
Good risks	3 of 43 = 9 5	3 of [illegible]7 = 11 1
Bad risks	10 of 94 = 10 6	15 of 67 = [illegible]4

Table IX *Thrombo embolic episodes in the long term groups*

	Anticoagulant treated	Placebo treated
Recurrence of myocardial infarction	9 recurrences in 7 patients (5 †)	19 recurrences in 18 patients (15 †)
Pulmonary embolism	1 patient (1 †)	
Thrombophlebitis		1 patient
Peripheral arterial embolism		1 patient
Total	10 episodes in 8 patients	21 episodes in 20 patients

rences appear to be evenly distributed over the two year observation period

Table IX shows that thrombo embolic episodes other than recurrence of myocardial infarction were few

Haemorrhagic complications occurred in 22 2 % of the anticoagulant treated patients in the long term group Using the same criteria as in the short term material we found 25 episodes of mild bleeding in 24 patients and nine episodes of severe bleeding in eight patients Thus severe bleeding occurred in 5 7 % of the patients

One patient died of bleeding from a duodenal ulcer with haematemesis Autopsy also showed a large retroperitoneal haematoma The patient had bled for several days and died a few minutes after admission The prothrombin value was not obtained

In the placebo group mild bleeding occurred in one patient (1 1 %)

DISCUSSION

On the basis of the findings presented here an attempt will be made to appraise the efficacy of anticoagulant treatment of myocardial infarction in the acute stage (short term material) as well as during the subsequent period of up to two years (long term material)

The short term material comprised two fully comparable groups 156 anticoagulant treated patients and 120 placebo treated patients Anticoagulant treatment was shown to result in

1 significant reduction of thrombo-embolic complications
2 significant reduction of the mortality rate
3 significant increase in haemorrhagic episodes

All authors referred to (5 8 9 10 13 14 18 21 22) except Feldman et al (4) and Wasserman et al (20) agree that anticoagulant treatment reduces the number of venous thrombo-embolic complications The present material also shows a marked tendency towards a reduction in the incidence of cerebral thrombosis as was also observed by Harvald et al (6) in their long term study Our figures are so small however that the finding may be fortuitous

There is some divergence of opinion concerning the effect on the mortality rate Feldman et al (4) Hilden et al (8) and Wasserman et al (20) found no effect whereas Wright et al (21 22) Tulloch and Gilchrist (18) Holten (9) Honey & Truelove (10) McCluskie & Seaton (13) Griffith et al (5) and Rashkoff et al (14) observed a conspicuous reduction in the mortality rate as in the present study

Some of the authors mentioned found that the reduction in the mortality rate is significant only in some subgroups of their material thus Tulloch and Gilchrist (18) in male patients Holten (9) in patients between 60 and 69 Wright et al (21 22) in patients over 60 McCluskie and Seaton (13) in patients under 60 and Griffith et al (5) only in patients under 70 in the bad risk group

When the present material was subdivided according to sex age groups or good and bad risk criteria none of these subgroups could be shown to profit especially by anticoagulant treatment In obese patients and/or diabetics on the other hand the difference between the anticoagulant treated and the placebo-treated is much greater than in the material as a whole both in regard to mortality rate and thrombo-embolic complications

The reduction of the mortality rate is mainly due to the decreased number of thrombo-embolic complications the difference is practically eliminated when deaths due to thrombo-embolism are excluded

The number of haemorrhagic complications is

of the same magnitude as that found by Feldman et al (4) Griffith et al (5) Rashkoff et al (14) and Holten (9) whereas the materials of Wright et al (21 22) and Hilden et al (8) show a higher incidence of bleeding In view of the reduction in mortality rate and thrombo-embolic complications obtained by anticoagulant treatment we do not consider that the occurrence of severe bleeding in three patients with two deaths neither of which could for certain be ascribed to haemorrhage offers sufficient grounds for abandoning this treatment.

The long term groups comprising 139 anticoagulant treated and 95 placebo-treated patients are fully comparable except for the number of patients in the two groups compared with the number originally admitted The difference which is statistically significant is due to an excess of deaths in the placebo-group in the first 4 weeks after the infarction.

In the long term material the following effects of anticoagulant treatment were observed

1 a significant reduction of the mortality rate in patients originally regarded as bad risks
2 a significant reduction in the rate of recurrence in patients over 60
3 a significant increase in the number of haemorrhages

Whereas The Medical Research Council (15) Clausen et al (3) Harvald et al (6) and Seaman et al (17) did not observe that treatment had any effect on the mortality rate Manchester (11 12) found a considerable reduction in the mortality rate Bjerkelund (2) a reduction in the mortality rate in men under 60 but only during the first year of treatment The Veterans Administration Report (19) stated that there was a reduction in the mortality rate restricted to patients under 55 which was most pronounced during the first two years of treatment

All authors except Seaman et al (17) considered that anticoagulant treatment has a favourable influence on the recurrence frequency in some categories of patients Thus Bjerkelund (2) observed this in patients under 60 Clausen et al (3) Medical Research Council (15) and The Veterans Administration Report (19) in patients under 55 Harvald et al (6) in patients under 60—though when the same material was re-evaluated after a further two years the effect was apparent only in patients over 60 (7) and Aspenstrom (1) in patients over 60

The present material shows an effect in patients over 60 It is evident, however from the discrepancies cited above that, though there may be an actual effect, it is nevertheless small and the materials hitherto reported on afford an insufficient basis for a final decision

The number of haemorrhages during long term anticoagulant treatment varies widely in different studies from about 3 to 80–90° To some extent this variation is probably due to differences in the care with which mild episodes have been recorded and to differences in the length of the observation periods

The present study does not answer the question, for how long after the acute stage does the treatment continue to have an effect on the mortality and recurrence rates Bjerkelund (2) Clausen et al (3) and Harvald et al (6) came to the conclusion that treatment is effective only for the first $^1/_2$–1 year They found that then the difference between the anticoagulant treated patients and the placebo group had levelled out.

CONCLUSION

Our study indicates that anticoagulant treatment has some effect on myocardial infarction both in the acute stage and during the subsequent two years Against this must be weighed the risk of bleeding which is connected with treatment and one must consider whether a favourable effect might be attained by less hazardous means For instance the use of a more liberal regimen in the acute stage following myocardial infarction may lessen the number of thrombo-embolic complications and thereby reduce mortality

We would conclude as follows

1 All patients with myocardial infarction should be given anticoagulant treatment in the acute stage—at least if and for as long as strict bed rest is applied With a more liberal regimen it may be sufficient to treat only such patients in whom early mobilization is not feasible In the case of diabetes and/or obesity anticoagulant treatment should be given

2 It is far more difficult to decide what categories of patients should have long term anticoagulant treatment and for how long When we compare our findings with those of others ob-

tained in materials of similar magnitude the results vary so widely that we cannot with confidence point to particular groups who should preferentially be given long term anticoagulant treatment.

We believe nevertheless that our study has demonstrated that some patients do benefit from long term treatment But it must be emphasized that this form of treatment demands frequent and careful control and should be reserved for patients who are willing and able to cooperate

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HODGKIN S DISEASE IN NORWAY

Mortality Trends Incidence and Survival

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Abstract Recorded mortality from Hodgkin s disease in Norway since 1931 showed at ages less than 50 years an increase until 1951–1955 and at ages 50 and over an increase throughout the period 1931–1965 The main features of the trends in recorded mortality are compatible with the effects of improved possibilities for the recognition of the disease

A series of 870 cases of Hodgkin s disease notified to the Cancer Registry of Norway as diagnosed during the period 1953–1963 is reported on. All cases were histologically diagnosed.

Age-specific incidence rates of Hodgkin s disease exhibit a characteristic bimodal curve Both in males and females the incidence rates increased rapidly from age 10–15 and reached a peak at age 20–30 After a e 40–50 the incidence started increasing again with increasing age From age 20 the incidence was higher in males than in females. For malignant lymphomas as a group—and the findings for Hodgkin s disease were in line with those for the other lymphomas—the incidence at ages less than 55 was higher in single women than in women who had ever married.

A grouping by county of residence did not reveal any marked geographical differences in average incidence No special seasonal pattern in the occurrence of Hodgkin s disease was detected. The distribution of cases by county of res dence and time of diagnosis (2 year intervals) suggests a clustering of newly diagnosed cases in time and space

For cases diagnosed in 1953–1958 relative survival rates were highest in patients less than 65 years of age For the age group less than 45 the 5 year survival rate from the time of diagnosis was 30 percent for males and 44 for females

The following report on the occurrence of Hodgkin s disease in Norway is presented in the hope that it may help furnish a basis for the formulation of hypotheses as to etiology and pathogenesis The data are derived from the official mortality statistics and the Cancer Registry of Norway Such data lack some of the details one might desire in assessing various aspects of the disease e g survival However in contrast to data on patients seen in a particular hospital by referring to a defined population and thus depicting the community experience a more valid basis for generalization is ensured

SOURCES OF DATA

Age and sex-specific mortality rates from Hodgkin s disease for the various 5 year intervals of the period 1931–1955 have been published by Backer (4) Supplementary mortality rates for the periods 1956–1960 and 1961–1965 were computed from data in annual reports from the Central Bureau of Statistics (17 18 19)

The morbidity data are derived from the Cancer Registry of Norway The Cancer Registry covers the total population of Norway and is based on reports of all new cases of cancer and allied diseases. A detailed description of the registration scheme has been gi en elsewhere (21) It is assumed that the completeness of registration of recognized cases is very high

The material to be presented comprises all new cases of Hodgkin s disease (ISC 01) currently recorded in the Registry as having been diagnosed during the years 1953–1963 For the computation of incidence rates person years at risk were estimated from population data as of Dec 31 1955 and Nov 1 1960 (20) The cases diagnosed in the period 1953–1958 and recorded in the Registry as of September 1 1961 were followed up through July 1 1961 The survival analysis covers this group of patients in whom a minimum of 2 years of follow up was available

RESULTS

Mortality

In the 5 year period 1961–1965 Hodgkin s disease was recorded as the underlying cause of 223 deaths among males and 149 deaths among females i e 45 deaths among males and 30 deaths among females per year The average annual death rate was 24 per million for males and 16 per million for females

Table I *Average annual death rates from Hodgkin s disease per million population Norway 1931–1965*

Sex and period	Age (y) 15–19	20–29	30–39	40–49	50–59	60–69	70–79	80 and over
Males								
1931–35	3	7	5	12	14	12	24	—
1936–40	8	11	13	14	19	33	19	43
1941–45	6	16	13	14	24	20	39	10
1946–50	9	12	18	20	24	31	41	6
1951–55	10	14	31	27	27	34	43	49
1956–60	2	25	22	22	36	47	57	70
1961–65	10	21	26	36	44	32	66	72
Females								
1931–35	3	5	8	7	9	18	22	—
1936–40	3	5	12	7	4	15	24	15
1941–45	—	11	13	9	13	15	20	14
1946–50	6	22	18	13	15	21	26	25
1951–55	2	20	15	18	18	38	37	24
1956–60	2	8	21	21	23	37	33	26
1961–65	8	15	15	14	21	37	39	57

Age and sex specific death rates from Hodgkin s disease for the seven 5 year periods from 1931 to 1965 for persons 15 years of age and older are given in Table I For each period a

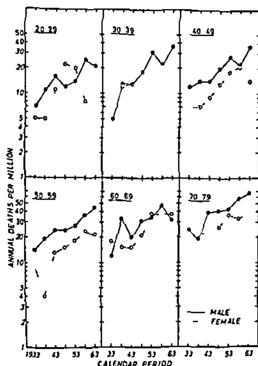


Fig 1 Trends in average annual age specific mortality rates from Hodgkin s disease by sex Norway 1931–1965

tendency is observed for the death rate to increase with age In each age group the death rate was higher in 1961–1965 than in 1931–1935

Fig 1 shows the trends in mortality between 1931 and 1965 for six 10 year age groups The midpoint of each 5 year calendar period is indicated on the abscissa The age groups less than 50 (the three upper panels) experienced a rise in mortality up to the period 1951–1955 with no clear evidence of increase after that period For the age groups over 50 (the three lower panels) increasing mortality can be observed throughout the period 1931–1965 In each age group mortality was in general higher among males than among females

Morbidity

In the Cancer Registry of Norway 2394 cases of malignant lymphoma (ISC 200–202) are recorded as newly diagnosed during the years 1953–1963 (Table II) For a case to be classed as Hodgkin s disease a histologic diagnosis of Hodgkin s disease was required Cases with a clinical diagnosis of Hodgkin s disease but with an equivocal histologic diagnosis such as malignant lymphoma or with reports from different pathologists diverging as to type of lymphoma were classed with other and malignant lymphoma not further specified" Similar rules applied to cases of reticulosarcoma and lymphosarcoma Thus the cases classed as either Hodgkin s disease reticulosarcoma or lymphosarcoma were all diagnosed histologically For more than 97 percent of the total malignant lymphoma cases the diagnosis was confirmed by histologic examination

The annual number of new cases of Hodgkin s disease increased slightly during the years 1953–1963 mainly due to an increase in the number of

Table II *New cases of malignant lymphoma (ISC 200–202) by sex and histologic type Norway 1953–63*

	♂	♀	Total
Hodgkin s disease	479	341	8 0
Reticulosarcoma	337	240	577
Lymphosarcoma	301	168	469
Other and malignant lymphoma" not further specified	314	214	5 8
Total	1431	963	2394

new cases recognized in old persons During the last part of the period on average 50 new cases among *males* and *33 new cases among females* were diagnosed annually This corresponds to an nual incidence rates of 28 per million and 18 per million respectively i e somewhat higher than annual mortality of the disease in 1961–1965 To secure fairly stable rates the material for the total period 1953–1963 was used in the calculation of various age specific incidence rates

Age

The *age specific incidence rates of Hodgkin s* disease 5 year age groups exhibit a characteristic bimodal curve Fig 2 which shows the incidence in males illustrates how the incidence increases rapidly from age 10–14 and reaches a peak at age 20–29 Thereafter there is a slight decrease until the incidence starts increasing again at older ages In reticulosarcoma and lymphosarcoma the in cidence increases at a fairly constant rate with in creasing age In absolute level as well as in form the incidence curve for other and malignant lymphoma not further specified (not charted) would conform closely with those of reticulo sarcoma and lymphosarcoma

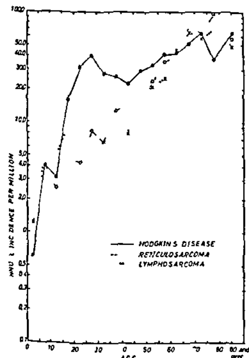


Fig Average annual age-specific incidence rates of Hodgkin s disease reticulosarcoma, and lymphosarcoma in males Norway 1953–1963

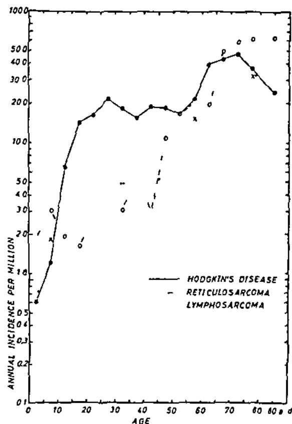


Fig 3 Average annual age specific incidence rates of Hodgkin s disease reticulosarcoma and lymphosarcoma in females Norway 1953–1963

The incidence rates for females (Fig 3) display a similar picture In Hodgkin s disease the in cidence increases rapidly until age 20–29 then shows a plateau succeeded by higher rates at ages over 60 In reticulosarcoma and lymphosarcoma on the other hand the rate of increase of in cidence with increasing age is in the main con stant

Sex

In the left panel of Fig 4 the age incidence curves for Hodgkin s disease in males and in females can be compared At ages less than 15 the incidence was the same in the two sexes In the age group 15–24 the rates show a male excess of 50 percent and in each of the ensuing age groups there is a male excess of about the same order of magnitude The right panel of Fig 4 shows age and sex specific incidence rates for all other malignant lymphomas combined At all ages the rates in males are more than 50 percent higher than in

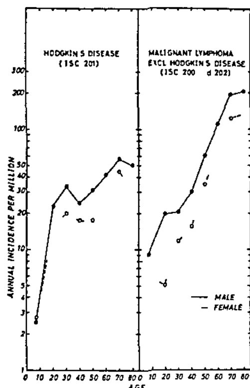


Fig 4 Average annual age specific incidence rates of malignant lymphoma (ISC 200–202) by type of lymphoma and sex Norway 1953–1963

females After age 35–44 there is however a slight but consistent reduction of the sex ratio with increasing age

Marital status

Fig 5 shows the age incidence curves for women over 20 years of age by marital status Since the never married group was relatively small at most ages and the rates consequently subject to large random errors one should not place too much emphasis on differences in rates between the two groups of women in Hodgkin s disease One can see however that the rates for never married are slightly higher than the rates for married and formerly married before age 55–64 a feature which is more pronounced for all other malignant lymphomas combined

Time

The distribution by month of birth of the cases of Hodgkin s disease diagnosed in 1953–1963 did not deviate appreciably from expectation on the basis of the monthly distribution of births during the relevant years of birth If there were a seasonal trend in the onset of the disease one might expect this to be reflected in the distribution of cases by month of diagnosis Between relatively wide age intervals there were some differences in the distribution of cases by month of diagnosis Considered as a whole however variations in the distribution of cases by month of diagnosis could easily be ascribed to chance In particular no seasonal trend was observed for those aged 15–34 i e the age group contributing to the first peak of the age incidence curve

Place

The recorded incidence of Hodgkin s disease was higher in urban areas than in rural areas and in rural areas the incidence appeared higher in densely populated than in sparsely populated areas This applied to both sexes also when the analysis was restricted to persons less than 70 years of age In view of the association of recorded incidence with urbanization the observed numbers of new cases in persons less than 70 in the individual counties showed no striking deviations from expectation on the basis of national rates

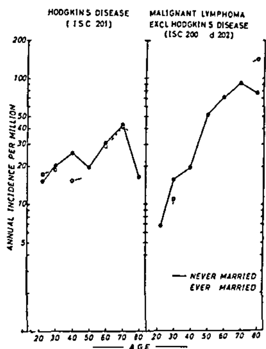


Fig 5 Average annual age-specific incidence rates of malignant lymphoma (ISC 200 20?) among women by type of lymphoma and marital status Norway 1953–1963

Time and place

A crude attempt to identify time space aggregation was made by cross classifying the cases (both *sexes and all ages combined) by time of diagnosis (? year calendar periods)* and by county (Table III) To avoid expected frequencies less than 5 in any cell contiguous counties with few cases were combined Assuming which cannot be entirely wrong that changes in composition and size of the population in the individual counties over the 10-year period were of negligible consequence the data do suggest time space aggregation of new cases of Hodgkin s disease A more penetrating analysis of the data with respect to time space clustering is intended

Site of affection

In 45 percent of the cases involvement of two or more anatomical regions was noted at the time of diagnosis (Table IV) This percentage showed only minor variation with age Of the 420 cases in which the affection apparently was limited to a single anatomical region 213 or 51 percent had involvement of cervical nodes Abdominal and retroperitoneal involvement was noted in 17 per cent of the cases with affection limited to a single anatomical region

Table IV sets out the numbers for three age groups The age group less than 45 consists mainly of cases contributing to the first peak of the age incidence curve and the age group 65 and over corresponds to the second peak The preponder

Table III *Number of new cases of Hodgkin s disease by county of residence and time of diagnosis (2 year periods) Norway 1953–1962*

$\chi^2 = 77\ 07$ 52 d f $0\ 01 < P < 0\ 0?5$

County of residence	Time of diagnosis 1953–54	1955–56	1957–58	1959–60	1961–62	Total 1953–62
Østfold	3	3	9	15	7	37
Akershus	12	4	9	14	6	45
Oslo	19	18	23	39	27	1?6
Hedmark	4	11	12	8	9	44
Oppland	12	3	11	12	13	51
Buskerud	17	7	4	5	6	39
Vestfold	8	4	7	4	10	33
Telemark	4	9	8	5	10	36
Aust and Vest Agder	9	8	4	12	10	43
Rogaland	*6*	*8*	*6*	*11*	*11*	*42*
Hordaland and Bergen	10	10	8	4	18	50
Sogn & Fjordane and Møre & Romsdal	14	12	7	10	16	59
Sør and Nord Trøndelag	10	14	10	18	15	67
Nordland Troms and Finnmark	18	16	12	0	15	81
The whole country	146	1?7	130	177	173	753

Table IV *New cases of Hodgkin s disease by site of affection as noted at time of diagnosis age and sex Norway 1953–1963*

Site of affection	0–44 y ♂	♀	Sexes comb No ( )	45–64 y ♂	♀	Sexes comb No ( )	65 y and over ♂	♀	Sexes comb No ( )	All ages ♂	♀	Sexes comb No ([a])
Cervical	74	44	118 (62 8)	28	34	62 (43 1)	15	18	33 (37 5)	117	96	213 (50 7)
Intrathoracal	11	16	27 (14 4)	10	5	15 (10 4)	4	3	7 (8 0)	25	24	49 (11 7)
Abdominal retroperitoneal	6	4	10 (5 3)	26	7	33 (22 9)	12	17	9 (33 0)	44	28	72 (17 1)
Axillary lymph nodes of upper extremities	11	3	14 (7 4)	13	7	20 (13 9)	5	3	8 (9 1)	29	13	42 (10 0)
Inguinal lymph nodes of lower extremities	6	6	12 (6 4)	4	1	5 (3 5)	3	—	3 (3 4)	13	7	0 (4 8)
Other	2	5	7 (3 7)	4	5	9 (6 3)	5	3	8 (9 1)	11	13	24 (5 7)
Site not specified	9	3	12	4	1	5	6	4	10	19	8	27
Multiple sites	104	65	169	67	46	113	50	41	91	2 1	152	373
Total	223	146	369	156	106	262	100	89	189	479	341	8 0

[a] Percent of those with affection limited to one site as noted at time of diagnosis

Table V *Five year survival rates for patients with Hodgkin s disease by sex and age at diagnosis Norway 1953–1958*

Age (y)	Sex	No of cases	Five year survival rates ( ) Observed	Expected	Relative
0–44	♂	113	29 5	99 2	29 7
	♀	77	44 1	99 5	44 3
45–64	♂	67	26 8	93 8	28 6
	♀	55	32 8	96 0	34 2
65 and over	♂	39	10 3	69 9	14 7
	♀	34	11 8	80 4	14 7
All ages	♂	219	25 3	92 0	27 5
	♀	166	33 8	94 3	35 8

ance of cases with cervical involvement is most pronounced at younger ages and decreases with increasing age Abdominal and retroperitoneal involvement on the other hand appears to be relatively more frequent at older ages

A similar tabulation of the reticulosarcomas and the lymphosarcomas showed a higher proportion of cases with apparently localized disease at the time of diagnosis Among localized cases the frequency of cervical involvement was lower and frequency of abdominal and retroperitoneal olvement higher than in Hodgkin s disease These frequencies did not vary appreciably between the three age groups

Survival

The survival analysis was confined to cases diagnosed during the years 1953–1958 After the survival analysis had been carried out a few additional cases of Hodgkin s disease were recorded as diagnosed during the period covered by the analysis A more detailed analysis of survival including the more recently recorded cases is contemplated It is contended however that the results presently available give a valid reflection of the dependence of survival on sex and age

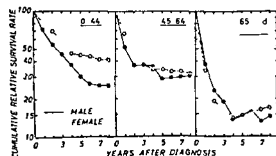


Fig 6 Relative survival rates (%) 0–8 years after diagnosis for patients with Hodgkin s disease by age and sex Norway 1953–1958

The cases were divided by sex and age Since the risk of dying from causes other than Hodgkin s disease varies with sex and age relative survival rates were estimated to enable a meaningful comparison of the severity of the disease in the various sex/age groups The relative survival rate is estimated as the ratio of the observed survival rate to the expected rate for a group of persons in the general population similar to th patient group with respect to sex age and calendar period of observation (9)

The 5 year relative survival rate (Table V) was lower at age 65 and over than in the younger age groups In the age group less than 45 the survival rate was higher in females than in males The curves depicting the cumulative survival rates (Fig 6) level off 5 to 7 years after diagnosis suggesting that patients who have survived for at least 7 years after the diagnosis of Hodgkin s disease experience a force of mortality similar to that of a group of persons in the general population with the same sex and age characteristics

DISCUSSION

Current mortality from Hodgkin s disease in Norway is essentially in accord with that reported for other European countries and North America (24) Secular changes in mortality have been modest and the increase observed for the various age groups after 1931–1935 has discontinued for all but those aged 70 and over The main pattern of the trends in age specific mortality is compatible with the effect of improved possibilities for recognition of the disease

Norwegian incidence rates conform rather closely with those reported from other European and North American cancer registries (12) The bimodality of the age incidence curve is a common feature and as can be observed in data from different calendar periods it can hardly be explained as a cohort phenomenon The age incidence curve for Hodgkin s disease in the 15–50 age range with a peak at age 20–30 is distinctly different from that of other malignant lymphomas Misclassification vis a vis other malignant lymphomas would if anything attenuate this characteristic

feature The age curve of Hodgkin s disease bears no resemblance to that of any other disease now classified as a malignant neoplasm.

A male to-female ratio in incidence of about 1 5 was found for each age group except the 0–14 group The rates at ages less than 15 were how ever based on few cases and would be very sensitive to chance variation. For comparison the unweighted means of the age and sex specific incidence rates for six European countries (excl Norway) Canada and the U S as estimated from cancer registry data (12) were calculated In this composite set of data a male excess was shown at all ages with the highest sex ratios (greater than 1 6) at ages 5–15 and 30–60

MacMahon (15) has put forward the hypothesis that what is now called Hodgkin s disease in cludes at least three subgroups which can be distinguished by age at clinical onset Apart from the bimodality of the age curve which distin guishes the three age groups 0–14 15–34 and 50 and over he listed differences in sex ratio and in international distribution as the most suggestive features Cancer registry data (12) do not re produce the pronounced changes in the sex ratio with age noted by MacMahon nor do they sup port his contention that certain European coun tries experience higher Hodgkin s disease rates under 40 years of age than the U S This does not invalidate the suggestion that consideration of in cidence in relation to age and sex may provide clues to etiology Another piece of information that might be helpful would come out of a study of the risk of Hodgkin s disease in women in rela tion to reproductive history The present series did not provide reliable information for an elu cidation of this point although the associated characteristic of marital status was recorded for most of the cases For malignant lymphoma as a group and the figures for Hodgkin s disease were in line with those for the other lymphomas the incidence in women who had never married was higher than in married or formerly married prior to age 55 Great caution should be exercised in the interpretation of this finding because of ran dom variation and possible bias due to informa tion on marital status being derived from dif ferent sources for the numerator and the de nominator of the rates Also it may be noted that the Ten City survey in the U S (7) showed a slightly higher incidence of cancer of the non reproductive organs among single women than among ever married before the age of 60

The 5 year relative survival rates for all ages combined given in this paper are identical with those reported by the End Results Group (10) summarizing the experience of more than 100 hospitals of various types and sizes in the U.S for cases diagnosed in the period 1950–54 The disease apparently runs a less severe course in women That the disease is less severe in persons under 45 than in older persons, and that the more favorable prognosis in females is restricted to persons less than 45 was also found in an un selected series from Saskatchewan (16) This con clusion draws support from various hospital series of treated patients as well (8 22) Although the follow up period here reported on was rather limited the shape of the survival curves are con sonant with Easson s (8) statement that survivors beyond 10 years should be considered cured

In patients presenting with apparently localized disease cervical nodes were the lymph node group most frequently involved Again an association with age was noted in that the relative frequency of cervical involvement in localized cases was higher in persons less than 45 years of age than in older persons The distribution by site of affec tion was not very different from the frequency distribution of the site of first d tected involve-ment reported by Smithers (23) which led him to suggest that the tumor initiating influences are not evenly distributed and that the regions drained by the cervical nodes provide the main source of the disorder

It is suggested that Hodgkin s disease may be looked upon as an age and sex-conditioned re sponse or end result of a response to some initiat ing stimuli Whether the condition will arise is determined by host factors such as immunologic reactivity and hormonal influences It would be illuminating if the results of tests illustrating the depression of delayed hypersensitivity found in early Hodgkin s disease (1) were described in rela tion to age and sex of the patients

Notable is the predilection for the 15–35 age group of certain diseases which on clinical and pathologic grounds may bear resemblance to Hodgkin s disease In Danish data (2) sarcoidosis which like Hodgkin s disease is associated with derangement of delayed hypersensitivity shows a pronounced peak in incidence at age 15–35 Th

berculosis was formerly frequently observed in association with Hodgkin s disease (13) In Norway the incidence of bacillary tuberculosis in the pe riod 1931 through 1955 was highest in persons between 20 and 30 years of age And age specific mortality rates from tuberculosis for individual cohorts which prior to 1951–55 had passed be yond the age of 20–30 demonstrated a peak at this same age (4) Infectious mononucleosis of suspected viral origin (14) has been noted to have occurred in conjunction with Hodgkin s disease in a number of cases (23) In a survey in Oxford (11) the incidence of infectious mono nucleosis with positive Paul–Bunnel reaction was found to be highest in the 20–29 age group

According to Burnet (3) many perhaps all non endemic infections show a peak of severity in young adults (15–35 years) Aisenberg (1) refer ring to evidence for depressed delayed hyper sensitivity during the acute stages of measles and other virus infections noted that these observa tions would be consistent with a viral causation of Hodgkin s disease

Cridland (6) found for a highly selected group of 106 cases of Hodgkin s disease constituting only 40 percent of the total cases seen in the particular hospital during the study period that 37 (35 percent) had a clinical onset in December or January Specification of the month of clinical onset is difficult and involves many uncertainties The month of diagnosis on the other hand can usually be fixed objectively If there were marked seasonal variation in the clinical onset of Hodg kin s disease one would expect this to be re flected in the distribution of cases by month of diagnosis The results of the present study are compatible with the assumption of no seasonal trend in the occurrence of Hodgkin s disease

The slight urban/rural differential in incidence rates exhibited by the present series may well be explained by differences in diagnosis Average in cidence of the disease during the study period showed only modest variation between individual counties

The cross-classification of cases by county of residence and time of diagnosis represents a crude approach to the study of time/space clustering of new cases of a disease With the chosen time/ space units which were to some degree determined due to ease of presentation and analysis the data do suggest that recognized cases of Hodgkin s disease tend to occur close together both in time and space Any far reaching interpretation is hardly warranted until a more detailed picture of this feature is available It is interesting to note that Clemmesen et al (5) in their Danish series from 1942–46 found a tendency toward greater variation in number of new cases from one year to another than would be expected on the assumption of constant risks in individual districts over the period

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WALDENSTRÖM S MACROGLOBULINAEMIA

An Unusual Case Having Only Pleuropulmonary Manifestations

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Abstract A case of Waldenstrom s macroglobulinaemia (W M) diagnosed by immunoelectrophoresis is reported Through 11 years the patient had been suffering from a gradually increasing pulmonary infiltration which for the past year or two had been accompanied by severe pleural effusion There were no other manifestations to indicate W M until the terminal stage when violent rectal haemorrhage occurred The diagnosis was confirmed by h stological examination which revealed infiltration by plasmo-lymphocytoid cells in the lung and pleura and also but in a minor degree in the liver kidneys spleen and lymph nodes Other reported cases of W M ac companied by pleuropulmonary affection are reviewed

Since Waldenstrom (20) in 1944 described three cases of macroglobulinaemia about 400 cases have been reported (1 7 9 11 15 17 18)

The disease manifests itself by the presence of abnormal proteins (macroglobulins) in the blood and the tissue fluids and by proliferation of cells which are often called plasmolymphocytoid re ticulum cells These cells infiltrate the reticulo endothelial system and histological changes are mostly found in the parenchymatous organs As changes affecting the lungs or pleura have seldom been described (3–5 9 11–18) we felt that a case in which the clinical changes were restricted to the right lung would be of interest

CASE REPORT

Male born April 7 1889 former school porter

Through many years the patient attended the annual prophylactic school examinations in the tuberculosis dispensary The X rays had been unremarkable until 1958 when the r ght hilar region showed a distinct en largement together with an infiltrate in the upper lobe No tubercle bacilli were found on this or other occasions The patient was feeling well and had no pulmonary complaints Follow up during the subsequent years showed the infiltrate to grow but little About 1965–1966 the enlargement ceased but now there was a large effusion in the right pleural cavity (For laboratory findings cf Table I)

In *November 1963* the patient was admitted for the first time to the Medical Department with an apoplectic attack Apart from mild expressive aphasia which dis appeared spontaneously neurological examination re vealed no abnormalities (B P 140/90 mm Hg) A straight chest film showed the right hilar region to be enlarged with large nodular borders and a shadow su esting atelectasis anteriorly and inferiorly in the upper lobe Bronchoscopy showed no ulceration or tumour tissue Biopsy from the carina showed normal conditions and the secretion did not contain malignant cells The pa tient asked to be discharged failed to attend follow up and was not seen until three years later

In *April 1966* he was readmitted after havin been suffering for 3 / months from dyspnoea fever and non sanguineous expectoration During the intervening three years he had been fit without any pulmonary symptoms Physical examination showed effus on in the right pleural cavity No enlargement of any lymph nodes liver or spleen could be demonstrated He was treated with antibiotics and thoracocentesis (yielding 1100 ml haemorrhagic serous fluid) (Fig 1) The fluid did not contain tumour cells or tubercle bacilli A thoracic surgeon who was consulted believed that the patient was suffering from an inoperable cancer of the lung How ever the patient refused to have further examinations owing to his advan ed age

In *June 1966* the patient was re admitted because of increasing dyspnoea through the past two weeks There was a large right sided pleural effusion which was re moved in three stages (totally 3350 ml flu d)

In *August 1966* the patient returned with ga troenteritis There was no manifest or occult bleeding 500 ml was evacuated again from the pleural space During this stay in hospital the ESR was 146 mm/h and paper electro phoresis on the serum showed a narrow tall peak in the gamma fraction (Table I) Exam nation for myelomatosis d d not confirm this d agnosis

Table I *Laboratory findings on admissions*

	1959 61	Nov 1962	1963 65[a]	April 1966	Sept. 1966	March 1967	June 1967	July 1967	Early Sept 1967	Late Sept. 1967	Normal values
Hb		100		85	63	72	62	68	64	50	100–125 %
R B C		4 2		3 8	2.9	3 6	2 9	2.8	2 2	2 6	5–5 5 × 10⁶/μl
Index col.		1 14		1 06	1 03	0 93	1 07	1 16	1 36	0 91	0 9–1 10
ESR	10	44	32/122	125	146	144	141	144	152	158	< 10 mm/1 h
W B C.		5900		6040	6300	9500	9800	5800	7400	6000	3–8.5 × 10³ μl
Diff		Norm		Norm	Norm	Norm	Norm	Norm	Norm	Norm	
Platelets				105 000	89 000	99 000	145 000	6500		6000	2–5 × 10⁵/μl
Crea		1.3		1 2		1.2	1 3	1 4	0 8	1 4	< 1 3 mg/100 ml
Protein				9 3	9 8	9 7	10 5	10 0	11 5	10 2	5 6–8 2 g/100 ml
Albumin					41	41	42	35	28		60–70 %
Alpha-1					1	2	3	2	1		2– 4
Alpha-2					5	6	8	2	3		4– 8
Beta					6	8	8	8	3		8–15 %
Gamma					47	43	39	53	65		10–18 %
Sia					Pos		Pos				

[a] Findings in the Tuberculosis Dispensary Other findings from the Department of Medicine

March 1967 Through four days he had had increasing cough dyspnoea and anorexia and was re-admitted, but thoracocentesis was not necessary

In *June 1967* the patient was re admitted with dyspnoea, sanguineous expectoration weight loss and severe fatigue Auscultation and X rays showed no new findings in the right lung and pleural cavity but the patient was in a poor general condition Previous studies had not demonstrated myelomatosis and now immunoelectrophoresis disclosed that the patient was suffering from macroglobulinaemia. Owing to the prolonged course of a tumour-suspicious pulmonary infiltrate this lesion was now suspected of being a link in the macroglobulinaemia. However a lung biopsy was not obtained because of the patient's poor condition He was given corticosteroid which improved him so much that he could be discharged for convalescence Two days later however he was admitted to another hospital with rectal haemorrhage which was treated with blood transfusion

In *September 1967* the patient was transferred to us from the other hospital. His anorexia and fatigue had further increased. He developed severe melaena and in spite of blood transfusions he deteriorated and expired a few days later

Laboratory findings

Table I gives the findings from all the admissions, while the following are from June 1967 Urine protein and urine electrophoresis negative Hydrophoresis (19) questionable positive Sia's test positive Immunoelectrophoresis appearances consistent with W M (IgM paraprotein with beta 2 mobility (J Clausen)) Sternal puncture greatly hyperplastic bone marrow with reduced normoblastic erythropoiesis. No signs of myelomatosis, W M or leukaemia. Chest radiography marked enlargement of the right hilar region, pronounced infiltration in the upper lobe and pleural effusion. X rays of the pelvis no abnormalities. B P 125/80 mm Hg. ECG no abnormalities From August 1966 there were changes indicating osteomalacia Serum calcium 7 4 mg/100 ml Serum phosphorus 2.4 mg/100 ml. Alkaline phosphatases 6–30 King Armstrong units. Urinary calcium 50–140 m / 24 h. The patient was examined for malabsorption but the xylose test, determination of fat and nitrogen in the faeces, oral glucose tolerance test, Schilling I test, serum B_{12}, and serum folic acid were normal GO-transaminases and icterus index also normal. Thymol 0 88 units. Takata Ara positive Serum cholesterol 80 mg/100 ml

Autopsy

Pleural cavities Strong adhesion in the middle of the right pleural cavity with some calcifications. *Trachea* Severe nodular infiltration of the mucous membrane which continued through the right main bronchus all the way down to the lower and upper lobes. *Bronchi* Mild stenosis of the bronchus in the 3rd segment of the right lung. *Intrathoracic lymph nodes* Soft, anthracotic Nothing to suggest metastatic infiltration *Lungs* The entire lower half of the right upper lobe was infiltrated with a strange solid pale reddish, slightly shiny tumour tissue of a uniform texture without any necroses. A streaky outrider from this tumour field ran along the pleura. This was the site of the extensive adhesion with calcific deposits. *Liver kidneys spleen abdominal lymph nodes* and *spine* No gross abnormalities. *Brain* Not examined.

Microscopic examination

Many samples of the *bronchus* showed it to be devoid of epithelium. The bronchial walls and the adjacent tissue showed a dense tumour like cellular infiltration almost obliterating the normal structure of the bronchial wall. Remnants of mucous glands only here and there

The infiltration consisted of clusters of lymphocyte like cells of varying size In these clusters also occasional plasma cells In addition, the dense infiltration contained major and m nor areas consisting of densely arran_ed, large cells which appeared to be plasma cells. Mild irregular fibrosis *The pu lmonary tissue from the af fected upper lobe* (Fig 2) had entirely lost its charac teristics in many sections The tissue was traversed by major and minor acellular hyalinized strands of con nective tissue between which were cellular inf ltrat ons as described above These infiltrations consisted in pla ces of lymphocyte like cells with very little cytoplasm in other places of plasma cells and lastly some sites gave the impression that the cells were reticular cell The main impression was an abnormal lymphoid tissue in large islets separated by connective tissue septa. *Lymph nodes f om the bifurcat on* (Fig. 3) consisted of tissue as in the upper lobe and the infiltration spread tumour like into the surrounding fatty and connective tissue *Hepatic t ssi e* The amount of connective tissue was slightly increased, showing in places plasma cells and lymphocyte like cells *Splenic tissue* Normal struc ture obliterated. In some places small, dense infiltrations similar to those in the lymph nodes In between ample accumulation of blood in the remnants of the pulp which incidentally contained numerous lymphocyte like cells and plasma cells The *bone ma row* showed infiltrates similar to those in the spleen *Renal tissue* Immediately beneath the capsule the cortex showed on cut section triangular infiltrates of the type like those in the other organs *Reticul n sta ning* of all the specimens was ne_a t ve in the infiltrates *PAS sta ning* was also negative In particular there were no nu lear changes with this staining. *Unna Pappenheim sta ning* All the preparations showed positive staining in the plasma-cell like areas of the infiltrations, and perhaps also in areas wh ch were interpreted as reticulum cells with ordinary staining. However the react on was everywhere fainter than shown by staining of control specimens containing "ordinary" plasma cells

Conclusion

The histological appearances are consistent with Waldenström s macroglobulinaemia The large tu mour like infiltrate in the pulmonary tissue and bronchi of the right lung must therefore be inter preted as a neoplastic manifestation of this disease

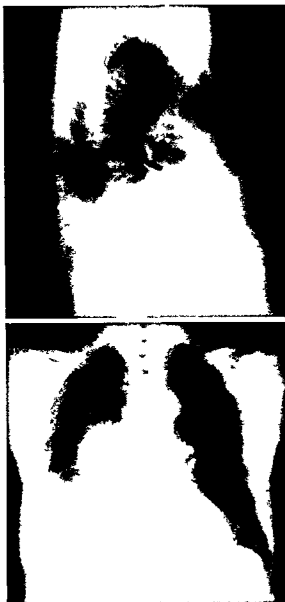


Fig 1 Enlargement of hilar region and pleural effusion (April 1966)

DISCUSSION

W M is a chronic proliferative disease of the reticulo-endothelial system affecting mainly el derly men

The most common symptoms are fatigue a haemorrhagic tendency visual disturbances dys pnoea, and repeated infections while the most common signs are hepato-splenomegaly lympha denopathy ocular changes as well as biochemical changes

The dyspnoea is often due to simple heart failure but the heart disease may be a link in the primary disease the macroglobulins increasing the viscosity of the serum and thus inhibiting cir culation X rays show in most cases only pulmonary congestion but there have been reports of pul monary and pleural changes in W M demon strated by X rays biopsy or autopsy (3–5 9 11–18)

Oettgen and Quitmann (15) (cases 2 and 3)

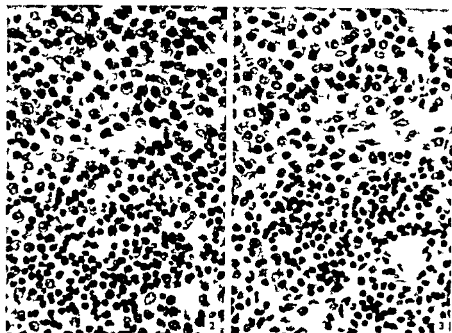


Fig 2 Pulmonary infiltration. Plasmocytoid cells (top) and lymphocytoid cells (bottom).

Fig 3 Lymph node Plasmocytoid cells (top) and lymphocytoid cells (bottom).

found peribronchial infiltration by lymphocytes and lymphoid reticulum cells Neither lesion had been diagnosed in vivo Schaumann (17) also made an accidental autopsy finding of a nodular accumulation of plasma cells in the lungs which he called pneumoplasmocytose micronodulaire Kappeler et al (9) in their case 9 found enlargement of the hilar glands and pleural effusion containing macroglobulins and in the sediment numerous small lymphocyte like cells McCallister et al (11) stated that two (cases 13 and 19) of their 31 cases had pleural effusion Fiere (4) found in three of his eight patients (cases 2 4 and 8) bilateral reticulonodular infiltrations Similar X rays findings have been described by Revol (16) in one of his patients However these two authors did not make any histological examination Furgerson et al (5) found delicate nodular infiltrations scattered in both lung fields Open lung biopsy disclosed pleural effusion The biopsy findings are not reported but subsequent autopsy revealed not only in the lungs but in a number of other organs an infiltration by plasmocytoid cells whose cytoplasm proved PAS-positive Moeschlin s (12) patient had miliary pulmonary infiltrates Biopsy revealed peribronchial and perivascular infiltration by lymphocytes and plasma cells as seen in W M Borch Nielsen and Bruzelius (13) patient had a small pulmonary infiltrate gradually increasing in size Histological examination of tissue removed in an exploratory thoracotomy showed numerous epithelioid-cell granulomas Autopsy a few years later when W M had been diagnosed disclosed lymphocytes and plasma cells but no epithelioid-cell reaction Espersen (3) found mild almost fibrous changes in both lungs but did not report a histological examination Schur and Appel (18) described a case in a 73 year-old man whose main symptom was dyspnoea because of pleural effusion which rapidly re accumulated after thoracocentesis The diagnosis of W M could be made on the basis of the effusion which upon paper electrophoresis showed a diagram corresponding to that found by electrophoresis on the serum Needle biopsy of the pleura also revealed lymphocytoid reticulum cells characteristic of W M Noach (14) was the first author to describe pleuropulmonary lesions in a patient with W M As in subsequent cases there were pulmonary infiltrates and pleural effusion on both sides

Thus the radiological as well as histological appearances have been rather varied. Only a few cases (13 14 and 18) have shown major lesions of the lungs and pleura as found in our case which differs also from the above mentioned cases in not having exhibited any of the symptoms or signs most often found in patients with W M Through several months our patient had had normochromic anaemia and elevated sedimenta

tion rate but this was put down to his presumed pulmonary carcinoma There was neither hepatosplenomegaly nor lymphadenopathy and it was not until the last month of life that he exhibited haemorrhagic diathesis Autopsy did not disclose any source of the haemorrhage

The case might have been a malignant pulmonary lesion accompanied secondarily by macroglobulinaemia Before 1965 there was a slow increment of the pulmonary infiltrate and the ESR too but from 1965/66 when the pulmonary lesion was accompanied by the large pleural effusion the ESR rose to very high values In retrospect it must be assumed that macroglobulinaemia was present at least at this juncture The prolonged course militates against carcinoma of the lung even though the enlargement of the infiltrate must be said to have been characterized by infiltrative growth Through 11 years the infiltrate was demonstrable without any pulmonary symptoms until the last year or two

The histological description of the cells in the examined organs of patients with W M has been that of atypical plasma cells and lymphocytes pervading the reticulo-endothelial system (7 9 10 11 15) It is characteristic that this pleomorphic cellular accumulation is demonstrable also in the bone marrow In our case a biopsy of the sternal bone marrow was obtained at a time of severe biochemical and radiological changes but no definite abnormalities were demonstrated After the autopsy the sternal bone marrow which had shown some reticular-cell proliferation in 1966 was re-examined Now we found a number of reticulum cells apart from a few atypical plasma cells and lymphocytes similar to the types of cell found in the lung

Further confirmation of the histological diagnosis was afforded by infiltration by plasmolymphocytoid cells in the kidneys spleen and liver in spite of the fact that these organs had not shown any gross abnormalities

The laboratory findings (cf Table I) were like those described by others Precipitation of protein made platelet count difficult Serum cholesterol was maybe low because of the W M or the osteomalacia

The pathogenesis of the disease has not yet been fully elucidated There is a cellular factor (2) as well as a biochemical factor (6) According to these studies the first process is a proliferation of the abnormal neoplastic type of cell and this is followed by the formation of macroglobulins The course in our case might perhaps be interpreted as a primary pulmonary lesion with subsequent development of symptomatic macroglobulinaemia There was no clinical evidence of a disseminated disease but histological examination did disclose that several parts of the reticulo-endothelial system were involved

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QUANTITATIVE ASPECTS OF LIPOLYSIS AND REESTERIFICATION IN HUMAN ADIPOSE TISSUE IN VITRO

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Abstract The capacity of human adipose tissue specimens, obtained during surgery to release glycerol and to reesterify free fatty acids was measured in vitro under different experimental conditions

Glycerol release was about 0.5 μmoles/g wet weight/h under basal conditions As compared with the epididymal fat pad of 250–350 g rats human subcutaneous adipose tissue showed a maximal lipolytic response to hormones which was only $/_{15}$ in young patients and $/_{30}$ in older patients Isolated human fat cells were not different from intact tissue Catecholamines gave higher lipolytic response than the peptide hormones ACTH glucagon and growth hormone with dexamethasone

Reesterification of free fatty acids as measured by the balance method was low in these fasting patients undergoing surgery It increased after addition of insulin or large amounts of glucose in vitro or after glucose infusion into the patient before surgery Free fat cells showed no difference compared with intact tissue Omental fat reesterified more fatty acids than subcutaneous adipose tissue

By parallel measurements of reesterification with the balance method and with glyceride synthesis from labelled glucose it was possible to show that in some cases, the latter method gave higher reesterification than the balance method This indicates a significant contribution of fatty acids from partial hydrolysis in the lipolytic process

These results show that triglycerides in human subcutaneous adipose tissue have a much slower turnover than in the rat epididymal fat pad This is more pronounced at a more advanced age which may contribute to the greater tendency to obesity with increasing age The results furthermore suggest that in these specimens taken from operated patients after 18 hours fasting, in spite of the low lipolytic potential reesterification of free fatty acids is so low that it cannot match lipolysis and fatty acid outflow increases An alternative explanation is partial hydrolysis of triglycerides

Studies on the metabolism of human adipose tissue in different clinical conditions require a detailed knowledge of the qualitative and quantitative characteristics during physiological conditions of the reactions to be investigated The necessity of this is stressed by the repeatedly noted species differences in adipose tissue metabolism which usually render impossible extrapolations from the adipose tissue metabolism of experimental animals known in detail to human adipose tissue metabolism (6 14 24 34) In a previous study it was reported that the lipid mobilization of human subcutaneous adipose tissue shows certain qualitative differences compared with that of the rat epididymal fat pad namely an increased ratio of free fatty acid (FFA) production/glycerol production after overnight fasting It could not be decided whether this increased ratio was due to a decreased reesterification or to retention of partial glycerides in the lipolytic or reesterification processes Certain quantitative differences between rat and human lipolysis were also indicated (6) The present paper deals in more detail with these questions from a quantitative point of view namely the lipolytic and reesterification capacity of human adipose tissue and the role of partial glycerides in these reactions

MATERIAL AND METHODS

Adipose tissue was obtained from patients operated on for different abdominal diseases None was icteric or grossly obese or had a malignant disease and none was in severe negative caloric balance according to hospital records. These patients fasted for 18 hours before operation, which was performed under general anesthesia after routine procedures with premedication of morphine or its analogues followed by Evipan (sodium hexobarbital Bayer Leverkusen, West Germany) nitrous oxide curarin, and diethyl ether administered during operation On the day before operation some of the patients as indicated in the Results section, received 300–400 g of glucose intravenously which was not continued longer than 5 hours before surgery Adipose tissue was removed as soon as possible after starting general anesthesia, and was taken

from the subcutaneous layer of the upper part of the abdominal wall or from the omentum majus The fat tissue was placed in Krebs-Ringer bicarbonate buffer with 4% bovine serum albumin (Armour Fraction V) pH 7 4 at room temperature and brought to the laboratory for immediate processing Incubation was started within 1 1/2 hours after removal preparation usually took about an hour

Techniques with Pieces of Adipose Tissue

Adipose tissue specimens of about 100 mg were prepared with tweezers and a pair of scissors avoiding too much handling of the tissue These pieces were then weighed on a torsion balance and incubated in 3 ml of Krebs Ringer bicarbonate solution with 4% bovine serum albumin with 10 mM glucose In one experimental series indicated in the Results section the incubation medium contained 85 mM glucose Gas phase was 95 % O_2-5% CO and final pH 7 4 Other conditions for incubation and measurements of total production of glycerol and FFA have been described previously (6) Fatty acid re esterification was calculated according to the balance method of Vaughan (35)

In certain series of incubations incorporation of glucose into glyceride glycerol and fatty acids of the glycerides was measured In these experiments $2 9 \times 10^6$ cpm of glucose U C (New England Nuclear Corp., Dreieichen lein, West Germany NEC-042H lot no 292–089 specific activity 9 5 millicurie/millimole) were added After termination of incubation of tissues and extraction of lipids an aliquot of the chloroform phase for fatty acid ermination was taken (3 15) and washed free from ter soluble radioactivity Part of this was evaporated 1 redissolved in 10 ml of 0 4% 2,5-diphenyloxazole (PPO) and 0 01% p-bis-2 (5 phenyloxazolyl) benzene (POPOP) (Packard LaGrange Illinois USA) in toluene Another part of the washed chloroform phase was evaporated and lipids saponified in an excess of ethanol potassium hydroxide for one hour at 60 C The solution was then acidified and fatty acids extracted into heptane and this phase was then counted as above Insignificant quenching was found to occur in connection with these counting procedures Total lipid radioactivity minus fatty acid radioactivity was then taken as glyceride glycerol radioactivity and this was expressed as radioactive glucose utilizing the counts obtained and the original specific activity of the incubation medium

In other experiments with radioactive glucose but not containing any non labelled glucose a suitable aliquot of the above mentioned washed chloroform phase was taken for thin layer chromatography of the lipids in the extract Silicic acid (Kieselgel H nach Stahl Merck Darmstadt, West Germany) was spread with a Desaga apparatus (C Desaga Heidelberg, West Germany) in a 0.25 mm layer over the glass plates, and dried at 1 0 C for an hour immediately before use Separation was obtained in heptane diethyl ether glacial acetic acid (85 15 . v v) in tightly closed chromatographic jars Human plasma lipids were run simultaneously as standard after previous identification of the lipid bands in this extract by utilizing commercial standards of cholesterol ester triglyceride fatty acid diglyceride cholesterol and lecithin. After subjecting the plate to iodine vapors for identification, bands on the adipose tissue chromatograms corresponding to plasma standard cholesterol esters, triglycerides, FFA, cholesterol + diglycerides and monoglycerides + phospholipids, were marked off After sublimation of the iodine at room temperature overnight the fractions listed were scraped off into counting vials, and counted as described by Snyder and Stephens (37) Digitonin precipitable lipids (33) contained only insignificant radioactivity tested in two experiments consequently the radioactivity of the cholesterol + diglyceride area was considered to be present in the diglycerides

Experiments with rat epididymal fat pads were performed with 250–350 g Sprague Dawley rats fed ad libitum, as described previously (4)

Techniques with Isolated Fat Cells

The technique described by Rodbell (27) for rat adipose tissue was utilized with some minor modifications. Ten mg of collagenase (type I Sigma lot no 105B 1640 and 66B-0630-1) were added to 6 ml of Krebs-Ringer bicarbonate solution with 4% bovine serum albumin and 10 mM glucose in a 25 ml siliconized Erlenmeyer flask. Approximately 3 g of fat in pieces of 50–100 mg, were added and incubated at 37 C in a 95% O_2-5% CO atmosphere final pH 7 4 and the flask shaken 120 times/min After two hours the contents were stirred with a plastic spatula and tissue fragments removed with tweezers The remaining turbid solution was poured into a translucent, plasti Spinco ultracentrifuge tube and left on the laboratory bench for about 10 min Fat droplets first floating to the surface were removed by aspiration Fat cells then floated to the top of the tube as indicated by a clear subnatant The latter was aspirated through a needle into a syringe and discarded The remaining cells were then washed in 4 ml of Krebs-Ringer albumin glucose solution by turning the tube upside down a couple of times and then again leaving the cells to float to the top This was repeated twice again after which the cells were suspended in the desired volume of incubation medium and distributed into siliconized or plastic incubation flasks with a siliconized pipette

Parallel experiments with rat epididymal fat pads and human subcutaneous adipose tissue gave a yield of fat cells from the human tissue of only about / of that from the rat tissue The washing procedure was found essential for in vitro responses of hormones Centrifugation of the free cell suspension even at very low g numbers, gave more breakage of human cells than of rat cells These experiences indica e that human fat cells are not as easy to remove from the adipose tissue stroma as rat epididymal fat pad cells are and as noted previously (10) the cells are probably more labile and easily damaged by mechanical trauma

Two ml of fat cell suspension in Krebs-Ringer bicarbonate albumin buffer were incubated in cylindrical plastic tubes Aliquots were taken for determinations of glycerol and FFA at the times indicated and after incubation also for determination of triglyceride contents (1) of each incubation flask Lipolysis in homogena es was measured as described previously (3 36)

Norepinephrine and epinephrine as bitartrates were obtained from Astra (Sodertalje Sweden) highly purified human growth hormone from Dr O Trygstad, Dept of Pediatrics Rikshospitalet Oslo Norway (cf 7) and dexamethasone from Organon (obtained from Pharmacia Uppsala Sweden) *adrenocorticotropic hormone (ACTH* batch 60723) from Ferring Malmo Sweden and glucagon (crystalline hydrochloride) from Eli Lilly Indianapolis Indiana, USA Growth hormone was dissolved in normal saline of pH 10 and dexamethasone in ethanol diluted to a final concentration of 0 5% ethanol in the incubation medium A corresponding blank was added to the other incubation flasks in the same series

RESULTS

Glycerol production and fatty acid reesterification as calculated by the balance method

Fig 1 shows the results of experiments with measurements of glycerol production and fatty acid reesterification calculated according to the balance method of Vaughan (35) Pieces of human subcutaneous fat showed only a moderate increase in glycerol production after the addition of a large amount of norepinephrine In the rat on the other hand a 15 fold increase was produced Fatty acid reesterification in human tissues was very small actually it was close to zero when calculated in this way An increase was obtained with insulin but not with norepinephrine In the rat tissues without addition of hormones reesterification was considerably higher than in human fat These results extend and confirm those previously reported (6)

Glycerol production in different adipose tissue preparations

In order to further investigate the small glycerol release from human adipose tissue compared with rat adipose tissue experiments were performed where this release was compared in free fat cells pieces of fat (slices) and in adipose tissue homogenates Fig 2 gives the results of one representative experiment In agreement with the results of Rodbell (28) free fat cells from rat adipose tissue showed a very high glycerol release after stimulation with norepinephrine considerably higher than the corresponding results with intact rat epididymal fat tissue or with rat fat homogenate With human subcutaneous fat however the glycerol production with free fat cells or with adipose tissue pieces was of the same magnitude

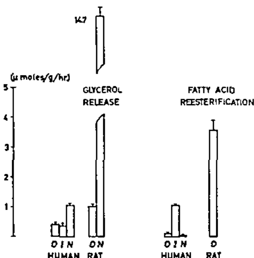


Fig 1 Glycerol release and fatty acid reesterification of human, subcutaneous adipose tissue and rat epididymal fat pad in vitro Means ± S E M of eight (human) and four (rat) experiments 0 no addition *I* insulin (1000 μU/ml) *N* norepinephrine (50 μg/ml)

In this particular experiment no increase of lipase activity was obtained after previous stimulation with norepinephrine a stimulation is otherwise the rule (cf 2 6)

Glycerol production with different lipolytic agents

Other agents known from other species to activate adipose tissue lipolysis were tried on the free fat cells of human subcutaneous adipose tissue The results of these experiments are shown in Fig 3 As with intact tissue (26) norepinephrine and epinephrine with and without caffeine were found more effective than glucagon or ACTH the latter showing no stimulating activity for certain Growth hormone with dexamethasone in concentrations shown to be optimal for lipolysis stimulation in rat fat cells by Fain et al (16) did not show any acute lipolytic stimulatory effect in human fat tissues (Fig 3) or after four hours of incubation with free fat cells or with pieces of fat tissue despite that these hormones produced a delayed lipolysis increase in flasks with rat fat run in parallel as control (7)

Glycerol production correlated with age and sex

Different correlations between age and glycerol and free fatty acid production in pieces of sub-

Fig 2 Glycerol and free fatty acid release from isolated fat cells tissue slices and homogenate from rat epididymal fat pad and human subcutaneous adipose tissue FFA, free fatty acids 0 no addition N norepinephrine (50 μg/ml)

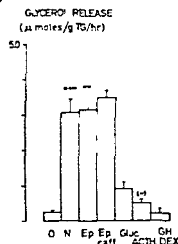


Fig 3 Effects of hormones on glycerol release from isolated, human, subcutaneous, adipose tissue fat-cells. Means ± S.E.M. of five experiments. 0 no addition N norepinephrine (50 μg ml) *Ep* epinephrine (50 μg ml) *Ep + caff* epinephrine (50 μg ml) plus caffein (13 mM) *Gluc* glucagon (12.5 μg ml) *ACTH* ACTH (7.5 IU ml) *GH + Dex*, growth hormone (1 μg ml) plus dexamethasone (0.016 μg ml). $P < 0.001$ $P < 0.05$ (*) $P < 0.10 > 0.05$

cutaneous adipose tissue were calculated A significant correlation was found between age and increased glycerol production on maximal norepinephrine stimulation of this release (Fig 4) Correlations were insignificant between age on the one hand and basal glycerol or free fatty acid production or total glycerol production or free fatty acid production after norepinephrine stimulation, as well as reesterification of fatty acids calculated according to the balance method (35) during basal and stimulated conditions. There were no apparent differences between men and women as exemplified in Fig 4

Reesterification of fatty acids under different conditions

Fig 5 shows fatty acid reesterification under different experimental conditions in human fat and in rat fat The small reesterification capacity in human subcutaneous adipose tissue during surgery and after a night's fasting was increased by the addition of either insulin in vitro or also large

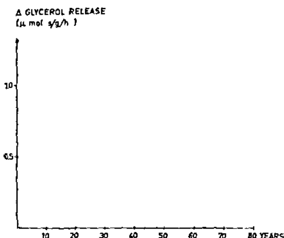


Fig 4 Increase of glycerol production from human subcutaneous ad pose tissue specimens at different ages after stimulation by norepinephrine (50 μg/ml) Circles = women. Crosses = men

amounts of glucose in vitro When the operated patients received 300–400 g of glucose intravenously on the day preceding surgery and continued up to five hours before operation reesterification was also increased Omental adipose tissue showed a higher reesterification capacity than subcutaneous fat Free fat cells from subcutaneous or omental fat showed reesterification which was not very different from that in intact tissue Data from the rat epididymal fat pad is added for comparison and are much higher than values for human subcutaneous fat

Comparison between reesterification values according to the balance method and after incorporation of label from glucose into glyceride glycerol

In fasting patients it is occasionally seen that there is very little reesterification of fatty acids when calculated according to the balance method In a series of samples however there is always some incorporation of label from glucose into glyceride glycerol When this figure is utilized for calculating reesterification, corrected for distribution in label in diglycerides (cf Table II) higher values are sometimes obtained than those given by the balance method This is exemplified in Table I where this discrepancy in the results has been listed as partial hydrolysis in patients no 1 and 5 during basal conditions and in patients no 3 and 5 after norepinephrine stimulation

Distribution of label from glucose in lipids

Table II gives the results of thin layer chromatography separations of the labelled lipids in subcutaneous adipose tissue under basal conditions and after stimulation with norepinephrine and insulin It may be seen that approximately $^2/_3$ of the total label are found in the triglyceride spot, while about $^1/_3$ is found in the diglyceride region under basal and stimulated conditions which confirms previously reported results (23)

DISCUSSION

Rodbell s (28) finding that rat adipose tissue lipolysis was substantially increased in isolated fat cells compared with intact adipose tissue is

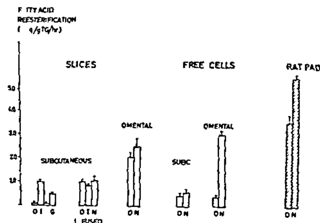


Fig 5 Reesterification of fatty acids in different preparations of human and rat fat tissues calculated according to the balance method (35) Means ± S.E.M. of 6–10 experiments. 0 no addition *I* insulin (1000 μU ml) *N* norepinephrine (50 μg ml) *G* glucose (85 mM) Infused = 300–400 g glucose given intravenously the day before sampling.

Table I *Balance between fatty acid and glycerol productions and glyceride synthesis in human subcutaneous adipose tissue*

Patient no	Basal 1	2	3	4	5	Norepinephrine (50 μg/ml) 1	2	3	4	5	Insulin (1000 μU/ml) 1	2	3	4	5
Tissue FFA concentrations 60[a] minus 0 (μEq/g)	0 78	−1 23	0 33	0	1 04	1 21	−0 51	2 12	0 74	3 37	−0 46	−1 14	−0 63	−0 32	−0 08
Medium FFA concentration 60 minus 0 (μEq/g)	0 32	0 53	1 15	0 59	3 83	1 95	1 00	1 51	0 64	3 96	0 17	0 53	0 21	0 07	1 57
Total FFA production (μEq/g)	1 10	0 70	1 48	0 59	4 87	3 16	0 49	3 63	1 38	7 33	−0 29	−0 61	−0 42	−0 25	1 49
Glycerol production (μmoles/g per 60)	0 47	0 31	0 73	0 45	1 53	1 29	0 53	1 32	0 75	2 42	0 44	0 33	0 61	0 26	0 71
Fatty acids reesterified according to balance method (μEq/g per 60)	0 31	1 63	0 71	0 76	−0 29	0 71	1 10	0 33	0 87	−0 07	1 61	1 60	2 25	1 03	0 64
^{14}C glyceride glycerol synthesis (μmoles/g per 60)	0 15	0 04	0 10	0 08	0 11	0 13	0 03	0 15	0 10	0 17	0 14	0 04	0 12	0 09	0 13
Fatty acids reesterified to ^{14}C glyceride glycerol[b] (μEq/g per 60)	0 41	0 11	0 27	0 22	0 30	0 35	0 09	0 41	0 27	0 46	0 38	0 11	0 33	0 25	0 39
Partial hydrolysis[c] (μEq/g per 60)	0 10	0	0	0	0 59	0	0	0 08	0	0 53	0	0	0	0	0

[a] Times given are after 30 preincubation
[b] Calculated from glyceride glycerol production and distribution of radioactivity in tri and diglycerides of Table II
[c] Fatty acids reesterified to ^{14}C glyceride glycerol minus fatty acids reesterified according to balance method (35)

Table II *Distribution of label from glucose U ^{14}C in human subcutaneous adipose tissue lipids*

	Basal	Norepinephrine (50 μg/ml)	Insulin (1000 U/ml)
Triglycerides	69 (60–73)	61 (52–63)	60 (60–62)
FFA	0	0	0
Diglycerides + cholesterol	22 (17–26)	32 (24–39)	38 (34–39)
Monoglycerides + phospholipids	9 (6–12)	7 (3–10)	2 (1–4)

Mean and range for four experiments

probably due to a decreased removal of fatty acids from the lipolytic compartment in the adipose tissue cell in the latter preparation Fatty acids have namely been shown to be powerful inhibitors of the lipolytic process in adipose tissue (1 13 28) The present finding that liberation of the fat cells from intact human adipose tissue does not increase the maximal lipolysis produced by norepinephrine seems most likely to be explained by the relatively slow breakdown of adipose tissue triglycerides to fatty acids in this tissue This process might well be so slow that even in intact tissue the concentration of fatty acids will not reach lipolytic inhibitory concentrations in the fat cells

Like adipose tissue from other species (29) human adipose tissue might rapidly inactivate lipolytic peptide hormones since as shown in the present work such hormones have a much smaller lipolytic stimulatory activity than catecholamines exert on isolated human fat cells which confirms similar data on intact human adipose tissue previously reported by Mosinger et al (26) This possible peptide hormone inactivating capacity of human adipose tissue in vitro does not however seem to be applicable to insulin since insulin has been demonstrated to be effective on both triglyceride synthesis and carbon dioxide formation from labelled glucose even in physiological concentrations (5 17 20 21 30) as well as an inhibitor of the lipolytic system (5 8)

The results in Fig 5 indicate that reesterification is low in human subcutaneous adipose tissue taken during surgery after 18 hours fasting Insulin or large amounts of glucose in vitro or glucose given before surgery in vivo apparently increase this capacity As seen in Figs 1 and 5

and demonstrated in a previous work (6) under these conditions of glucose abundance the magnitude of reesterification is such that it is able to retain fatty acids in subcutaneous adipose tissue and this is presumably the situation in the immediate postalimentary state After a relatively short fast human subcutaneous adipose tissue taken during surgical operations seems to release fatty acids through the absence of reesterification On the other hand when the rat has fasted for a period of time comparable with that of the operated patients its epididymal fat pad has a reesterification capacity under basal conditions in vitro which matches the lipolytic side much better than in human adipose tissue (35) In the rat stimulation of the lipolytic process seems necessary before lipolytic fatty acids can over flow the reesterification capacity and result in a net fatty acid release and lipid mobilization (37)

The reason for the deficient reesterification of fatty acids in the human tissues is not clear If fasting is responsible the lack of alpha glycerol phosphate precursors such as adipose tissue glycogen or glucose surrounding the fat cell seems a plausible explanation (cf 39) On the other hand it should be remembered that the surgical procedure as such constitutes a difference in conditions between the human and the rat experiments It is of interest that Jeanrenaud (25) has recently shown that glucocorticoids in physiological concentrations in vitro can selectively inhibit reesterification of fatty acids in adipose tissue without stimulation of lipolysis Glucocorticoid incretion is elevated in man on the day of surgical operation (18)

A major difficulty in interpreting the reesterification capacity of human adipose tissue is caused by the possibility that during lipolysis partial glycerides might accumulate on the lipolytic side Attempts have been made to measure the concentration of partial glycerides in human adipose tissue by thin layer chromatographic procedures and traces of such glycerides have been found It is however a spectacular technical problem to measure for example the small changes in diglyceride concentration that cause significant interference with the balance results This has been performed in the rat where the problem is easier because of a livelier lipolysis (31) In human tissues it would require for example the measurement of an increase in one gram of tissue from 25 to 25 5 μmoles of diglycerides which have to be separated from 1000 μmoles of triglycerides It has been considered impossible to do this with sufficient precision

There is however indirect evidence for the formation of partial glycerides on the lipolytic side in human subcutaneous adipose tissue as shown in Table I In certain cases the balance method does not register any reesterification but reesterification has occurred since labelled glycerides have been formed The formation of labelled glycerides is a minimum value due to unknown isotope dilution and therefore the reesterification is probably larger This discrepancy between the results of the balance method and those of the radioactive method is most readily explained by retention of partial glycerides on the lipolytic side Therefore such partial hydrolysis probably occurs in human adipose tissue to an unknown extent

In a recent work there was no evidence for retention of partial glycerides in incubated homogenates of human adipose tissue (6) This does not exclude such retention under conditions of an intact compartmentalization of glyceride pools in the fat cell Accumulation of partial glycerides has thus been shown to occur in intact adipose tissue viz in the perfused adipose tissue of the rat (31) as well as in the rabbit after catecholamine injection (38)

The results presented clearly demonstrate the poor lipolytic capacity of human subcutaneous adipose tissue compared with that of rat epididymal fat Particularly striking is the comparison between free fat cells stimulated by the most potent agent for producing increased glycerol release in human subcutaneous adipose tissue in vitro namely norepinephrine in a maximal dose Under these conditions glycerol release in the rat fat cells amounted to 15 times that in fat from a young man and even 50 times that in an old man

It is of interest to compare the quantitative data on in vitro lipolysis in human subcutaneous adipose tissue with the data on the turnover rate of plasma glycerol which is presumably a measure of mainly adipose tissue glycerol production The glycerol release in vitro from subcutaneous adipose tissue from patients who fasted overnight is approximately 0 5 μmoles/g/h Assuming a total adipose tissue weight of 10 kg this would

mean a glycerol turnover under basal fasting conditions of 83 μmoles/min which is in good agreement with the results obtained by Havel (22) in vivo During stimulation these figures are increased by a factor of 2–3 in both the in vitro and in vivo studies

It has already been suggested previously that the turnover rate of human adipose tissue triglyceride is relatively slow at least in comparison with that of the rat epididymal fat pad (6) This conclusion is strengthened by the present investigation Therefore on an average human subcutaneous adipose tissue may be considered as relatively static particularly with increasing age This inherent characteristic of human subcutaneous adipose tissue seems to be of considerable interest in regard to the tendency to obesity

It should be borne in mind however that this study does not exclude heterogeneity of the investigated adipose tissue samples so that certain fat cells may be much more active metabolically than the average values of the measured reactions in the cell population of an adipose tissue biopsy Such cell heterogeneity is suggested by recent studies of both glucose (30) and fatty acid metabolism (40) Half life of label from glucose in adipose tissue triglycerides in man also indicates a fat depot heterogeneity as regards both glucose uptake and lipid mobilization mechanisms which is perhaps due to different metabolic activities in different fat cells (9)

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SERUM FATTY ACID PATTERN IN CHRONIC ALCOHOLICS AFTER ACUTE ABUSE

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Abstract Serum lipids were studied in 14 chronic alcoholic males aged 30–56 years after a bout of drinking Ten male ward assistants aged 22–58 years, served as controls 1 No definite differences were found between alcoholics and controls in the level of total lipids and phospholipids in serum There was a significant increase of serum cholesterol in the alcoholics compared to the controls 2 Gas liquid, paper and thin layer chromatographic determinations of the fatty acid composition of serum cholesteryl esters demonstrated significantly lower figures for linoleic acid and significantly higher figures for monounsaturated fatty acids in the alcoholics 3 Gas liquid chromatographic determinations of the fatty acid composition of phosphoglycerides demonstrated that the decrease of linoleic acid and the increase of monounsaturated fatty acids were even more pronounced than in the cholesteryl esters 4 Five alcoholics who were restudied two weeks after admission showed a normalization of the fatty acid pattern The influence of diet and alcohol on the lipid pattern is discussed

The habitual abuse of alcohol may result in a variety of disorders such as acute alcoholic excitement acute alcohol hallucinosis Korsakow's psychosis alcoholic deterioration Wernicke's syndrome polyneuropathy and liver disorders These complications may be caused by a direct physical-chemical effect on the cell membrane or a changed cell metabolism due to the high alcohol intake However most of them are probably caused by a deficiency of essential nutrients (14) Deficiency of thiamine and/or other vitamins of the B complex group is widely believed to be the main culprit the next in order of importance being lack of essential amino acids Treatment usually consists of a diet rich in protein and carbohydrate fortified with extra B vitamins or a polyvitamin preparation

Since a high fat diet is believed to aggravate the liver injury caused by alcohol the need for an adequate intake of fat has not received the attention it deserves especially since some of the fatty acids are essential and have to be included in the diet and since the level of the essential fatty acids in the serum is abnormally low in the presence of liver disease (25 27) It was therefore considered legitimate to study the fatty acid pattern of the serum lipids in chronic alcoholics A deficiency of essential fatty acids may cause functional disturbances of the cell membrane in the brain and other organs and explain some of the symptoms of chronic alcoholism

In the present study we determined the fatty acid pattern of cholesteryl esters and phosphoglycerides by gas liquid chromatography in order to test the hypothesis that alcoholics have a deficiency of essential fatty acids In view of the evidence of a pronounced disturbance in the fatty acid pattern, a simple method for the clinical routine control of the serum fatty acid pattern was desirable Simple chromatographic methods were elaborated (22) and applied in the determination of the serum cholesteryl esters and the results were compared to those obtained by gas liquid chromatography

A preliminary report of the fatty acid changes of serum cholesteryl esters in the alcoholics has already been published (1)

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MATERIAL

The material consisted of 14 male alcoholics, aged 30–56 years (Table I) One alcoholic described in the preliminary communication (1) was excluded from the pres-

Table I *Clinical data of the 14 chronic alcoholics*

Case no	Age (y)	Advanced alcohol habits (y)	Last alcohol debauch Duration	Daily alcohol consumption (g)	Complications of alcoholism Earlier	Present	Highest value of serum glutamate oxalacetate transaminase value during present period
1	43	>20	1 week	400	Alcohol epilepsy Acute alcohol hallucinations		50
2	35	>15	1 month	200	Acute alcohol hallucinations		80
3	44	>10	3 weeks	300		Acute al ohol hallucinations	75
4	42	>20	1 week	300	Delirium tremens		
5	39	>20	1 month	300	Alcohol confusion	Acute alcohol hallucinations	30
6	43	>10	1 week	400	Delirium tremens	Acute alcohol hallucinations	38
7	56	>10	1 month	400	Delirium tremens		118
8	44	>20	3 weeks	300	Acute alcohol hallucinations		35
9	30	>10	3 weeks	300	Acute alcohol hallucinations	Delirium tremens	225
10	46	>25	3 days	200	Acute alcohol hallucinations		118
11	35	>15	1 week	300	Delirium tremens Alcohol epilepsy	Acute alcohol hallucinations	78
12	42	>20	8 days	300		Acute alcohol hallucinations	82
13	43	>20	1 month	300	Alcohol psychosis		30
14	56	>20	1 month	200	Acute alcohol hallucinations		143

ent study because of manifest diabetes diagnosed 3 months after he had left hospital They were all chronic alcoholics from a medical as well as from a social point of view Almost all of them belonged to the lowest socio economic class and five were known to be criminals (nos 2 4 5 8 and 12) They were all advanced gamma alco holics according to Jellinek s classification (13)

The duration of heavy habitual drinking and of the last bout are given in Table I The daily consumption of alcohol during the last period of abuse was reported as being between 200 and 400 g but was in reality possibly less In a larger Swedish series of alcoholics Goldberg (9) found the mean consumption to be 160 g/day In view of the excessive consumption of alcohol the intake of essential foodstuffs during the last 14 days before admission had certainly always been inadequate Several previous and present complications are given in Table I The serum glutamic acido-oxalacetic acid transaminase activity was used as a measure of liver function The highest values recorded while the men were in hospital are given in Table I

Five alcoholics (nos 1 7 11 13 and 14) were re examined two weeks after admission They had in the meantime been on an ordinary hospital diet consisting of 15 protein 50 carbohydrates and 35% fat of total calories All fat used in the preparation of food had a linoleic acid content of 15 These patients were given an extra supply of B vitamins

Ten male ward assistants aged 22–58 years served as controls They were all healthy and of normal weight They lived on an ordinary Swedish diet with at least one hot meal a day Three of them were teetotallers and the other seven consumed on the average at most 10 g alcohol a day Six controls described in the preliminary communication (1) were excluded from the present study because they did not fulfil the criteria for acceptance as controls

METHODS

Fasting venous blood samples were obtained within 3? hours of admission to hospital A total lipid extract of serum was prepared within 2 hours and stored in chloroform-methanol (2 1 v/v) at +4 until used As a rule chromatography was done within three days of collection of the blood and always within one week

Chomatographic separation of cholesteryl esters

Three portions of each extract were examined one was separated by paper chromatography (PC) the other two by thin layer chromatography (TLC)

A *Paper chromatography* A portion of the total lipid extract corresponding to 7 5 μl of serum was evap

Table II *Serum lipids in alcoholics and controls (mg % ± S.D.)*

	Total lipids	Cholesterol	Phospholipids
Alcoholics in acute period (n = 14)	753 ± 164	259 ± 46	289 ± 47
Controls (n = 10)	627 ± 156	206 ± 57	250 ± 53
p	$0.1 > p > 0.05$	$0.02 > p > 0.01$	$0.1 > p > 0.05$
Alcoholics (n = 5)			
in acute period	628 ± 98	220 ± 42	269 ± 63
after treatment 14 days	653 ± 105	228 ± 59	250 ± 23

p is determined from Student's t test for comparison between the groups of alcoholics (n = 14) and controls (n = 10)

orated in a stream of nitrogen, redissolved in 10 μl chloroform–methanol (2 : 1 v/v) and deposited with the aid of a micropipette as a 2 cm long band on a 19 × 19 cm Whatmann 3 paper impregnated with silicic acid according to Michalec (15) and described elsewhere (?). Ascending chromatography was done with light petroleum and the paper was stained with Rhodamine B. Quantitative determinations were made by scanning in a Vitatron densitometer. Filter 545. Every sample was scanned twice and the relative figures were calculated from the sum of the absorptions of the 5 cholesteryl ester fractions.

B. *Thin layer chromatography.* TLC was performed on plates coated with a 0.25 mm thick layer of Silica Gel G. A portion of the lipid extract corresponding to 20 μl of serum, was evaporated to dryness under a stream of nitrogen, then redissolved in chloroform–methanol (? : 1 v/v) and deposited as a 1.5 cm long band on the plate. The plates were developed with n-heptane–toluene (65 : 25 v/v) three times for 55 min each at 21°. One sample was stained with ammonium molybdate perchloric acid reagent, the other with potassium dichromate–50% sulphuric acid (19). Quantitative densitometry was done with the Vitatron apparatus. The filter was the same as that used for PC.

Gas liquid chromatography (GLC) of cholesteryl esters and phosphoglycerides

A portion of the lipid extract corresponding to 0.5 ml of serum, was separated on 0.5 g columns of silicic acid (Mallincrodt A.R., 120 mesh) into simple and polar lipids. The simple lipids were eluted with 10 ml of chloroform–methanol (50 : 1 v/v), the polar ones with 10 ml of methanol. The cholesteryl esters were separated from the other simple lipids by TLC on Silica Gel G by the method used for isolation of cholesteryl esters of tissues (3). Portions of 1/10 were used for GLC. The polar lipids were transmethylated with 0.1 N sodium methoxide in dry methanol for 60 min at room temperature and the methyl esters were isolated in the way described elsewhere (21). GLC was performed on stainless steel columns packed with 15% diethyleneglycol–succinate polyester (DEGS). The identification and quantification methods used have been described earlier (1).

Methods for quantitative lipid determinations

Total lipids were assayed by the phosphovanillin method described by Zöllner et al. (26), phospholipids by a modified Bartlett procedure, acyl glycerides (neutral fat) according to the method of Carlson (6) and total cholesterol according to the method of Crawford (8).

Statistical methods

Conventional statistical methods were used for calculating the mean and the standard deviation of the mean. The t-test was used for testing the significance of a difference when the groups were small (17). When the p-value was < 0.05 the difference was said to be significant.

RESULTS

Quantitative determination of total lipids, cholesterol and phospholipids

Table II gives the serum lipid levels in the controls and in the alcoholics after a drinking bout. No definite difference in total lipids and phosphoglycerides in the serum was found between the two groups. The cholesterol level was, however, significantly higher in the alcoholics.

A second set of samples obtained from five of the alcoholics after 14 days stay in hospital showed roughly the same pattern as the first set.

Fatty acid composition of cholesteryl esters (Tables III and IV)

Controls. Linoleic acid (18:2ω6), the major fatty acid in cholesteryl esters contributed 50%. GLC showed that monounsaturated fatty acids, mainly oleic acid (18:1) constituted about 25%. The concentration of saturated fatty acids was only about 15%. The concentrations found for polyenoic acid varied with the methods used, higher figures being obtained by the direct densitometric methods than by GLC. Arachidonic acid (20:4ω6) was the largest fraction of the polyenoic acids. Linolenic acid (18:3ω3) constituted only a minor portion of the acids in cholesteryl esters in serum. 22:6ω3 was not found.

Table III *Fatty acıds of serum cholesteryl esters ın 14 alcoholıcs (acute perıod) and 10 controls determıned by PC TLC and GLC*

Fatty acıds ( )	PC Mean±S D		TLC Am molybdate Mean±S D		TLC H_2SO_4 Mean ±S D		GLC	Mean±S D	
Controls									
C 0	14 1±1 8		13 7±2 2		12 9±3 0		14 0	1 3±0 4	
							16 0	13 5±1 6	
							18 0	1 3±0 3	
C 1	23 3±3 1		22 8±2 1		23 6±2 2		16 1	4 4±0 8	
							18 1	21 6±2 3	
C 2	49 4±3 1		51 4±5 2		50 2±4 0		18 2ω6	51 9±4 5	
C 3–4	10 6±1 0		9 6±2 4		9 7±1 6		18 3ω6	0 4±0 2	
							18 3ω3	0 7±0 2	
							20 3ω6	0 4±0 1	
							20 4ω6	3 4±1 6	
C 5–6	2 6±1 0		2 5±1 1		3 6±1 5		20 5ω3	0 9±0 5	
Alcoholics									
C 0	16 1±1 9	0 02>p>0 01	14 5±1 7	0 3>p>0 2	14 3±1 1	0 2>p>0 1	14 0	1 1±0 3	
							16 0	12 8±0 5	0 2>p>0 1
							18 0	0 8±0 2	<0 001
C 1	32 9±3 4	<0 001	32 5±3 5	<0 001	32 5±4 5	<0 001	16 1	8 6±2 5	<0 001
							18 1	26 8±4 1	0 01>p>0 001
C 2	37 9±3 9	<0 001	38 7±4 5	<0 001	39 5±3 6	<0 001	18 2ω6	41 9±5 7	<0 001
C 3–4	9 2±1 6	0 2>p>0 1	10 0±1 6	0 7>p>0 6	9 8±1 8	0 9>p>0 8	18 3ω6	0 5±0 3	
							18 3ω3	1 0±0 2	
							20 3ω6	0 5±0 1	
							20 4ω6	4 5±1 1	0 1>p>0 05
C 5–6	3 8±1 5	0 05>p>0 02	4 3±1 8	0 02>p>0 01	4 0±1 7	0 6>p>0 5	20 5ω3	1 6±0 8	

C 0=saturated C 1=monounsaturated C 2=dıunsaturated C 3–4=trı and tetraunsaturated C 5–6=penta and hexaunsaturated In GLC the values are gıven for correspondıng methyl esters p ıs determıned from Student s t test for comparıson between the groups of alcoholıcs and controls

Alcoholıcs The alcoholıcs dıffered from the two controls ın two major respects Fırstly lıno leıc acıd (18 2ω6) constıtuted only about 40% and by all the four methods ıt was found to be sıgnıfıcantly lower ($p<0\ 001$) than ın the con trols Regardıng other members of the lınoleıc acıd serıes the alcoholıcs dıd not dıffer from the controls Secondly monounsaturated fatty acıds (16 1 and 18 1) were ıncreased ($p<0\ 001$) ın the alcoholıcs GLC analysıs showed thıs ıncrease to be relatıvely larger for palmıtoleıc acıd (16 1) than for oleıc acıd (18 1)

Fıve alcoholıcs were re examıned after 14 days ın hospıtal The lınoleıc acıd content whıch was 38% on admıssıon then constıtuted about 47% of the fatty acıds ın cholesteryl esters whıch ıs only somewhat lower than ın the controls The monounsaturated acıds were also ıncreased shortly after admıssıon but now the fıgure for palmıto- leıc acıd was nearly normal whıle that for oleıc acıd was stıll ıncreased

Fatty acıd composıtıon of phosphoglycerıdes (Table V)

Controls Palmıtıc acıd (16 0) was the major fatty acıd ın serum phosphoglycerıdes and constıtuted 30% The monounsaturated fatty acıds constı tuted 15% and the proportıon between palmıto leıc and oleıc acıd was approxımately the same as that ın cholesteryl esters Lınoleıc acıd con stıtuted 26% Unlıke the cholesteryl esters the phosphoglycerıdes contaıned a consıderable amount of stearıc acıd (14 %)

Alcoholıcs The dıfference ın the dıstrıbutıon of fatty acıds between the alcoholıcs after a drınkıng bout and that ın the controls was even larger for phosphoglycerıdes than for cholesteryl esters Lınoleıc acıd (18 2ω6) constıtuted only 18% ın the alcoholıcs compared wıth 26% ın the controls ($p<0\ 001$) Monounsaturated fatty acıds (16 1 and 18 1) were sıgnıfıcantly ıncreased ($p<0\ 001$) ın the alcoholıcs No dıfference was found between the alcoholıcs and the controls re

Table IV *Fatty acids of serum cholesteryl esters in 5 alcoholics in acute period and after treatment for 14 days determined by PC TLC and GLC*

Fatty acids ( )	PC Mean±S.D	TLC Am molybdate Mean±S D	TLC H_2SO Mean±S D		GLC Mean±S D
Acute period					
C 0	15 8±1 2	14 4±0 8	14 7±1 2	14 0	1 1±0 2
				16 0	13 0±1 3
				18 0	0 6±0 2
C 1	31 7±3 5	33 9±3 1	33 7±2 6	16 1	10 1±2 6
				18 1	28 5±2 5
C 2	36 3±3 2	35 5±1 4	36 6±2 0	18 2ω6	38 0±2 7
C 3–4	9 7±3 1	10 9±1 0	10 8±0 5	18 3ω6	0 6±0 3
				18 3ω3	0 9±0 1
				20 3ω6	0 4±0 1
				20 4ω6	4 9±0 5
C 5–6	4 4±1 8	5 3±2 1	4 2±2 6	20 5ω3	1 9±0 6
After treatment 14 days					
C 0	16 7±2 8	13 6±1 3	14 8±1 3	14 0	0 8±0 3
				16 0	11 9±0 6
				18 0	1 0±0 5
C 1	27 5±2 5	26 4±3 5	26 7±4 1	16 1	5 3±2 4
				18 1	25 4±2 8
C 2	46 7±5 1	47 6±7 1	45 3±6 3	18 2ω6	46 8±5 2
C 3–4	6 7±2 2	9 0±2 5	8 9±1 4	18 3ω6	0 5±0 3
				18 3ω3	0 8±0 2
				20 3ω6	0 6±0 1
				20 4ω6	4 6±0 5
C 5–6	2 4±1 6	3 3±1 9	4 4±2 1	20 5ω3	1 4±1 1

Abbreviations of fatty acids as in Table III

garding saturated fatty acids (palmitic or stearic acid) Other fatty acids of the linoleic and linolenic series did not show any such significant difference either Five alcoholics who were restudied after 14 days in hospital had the same fatty acid composition in their serum phosphoglycerides in the acute period as the larger group of alcoholics except for linoleic acid which was still lower (16 %) Fourteen days later linoleic acid constituted 23 which is only somewhat lower than in the controls The monounsaturated fatty acid levels were not equal to those in the controls

Table V *Fatty acid composition of serum phosphoglycerides in 14 alcoholics (acute period) and 10 controls and in 5 alcoholics in acute period and after treatment for 14 days determined by GLC (values given in ±S D)*

	Controls (n=10)	Alcoholics (n=14)	p	Alcoholics (n=5) Acute period	Alcoholics (n=5) After treatment 14 days
16 0	31 4±4 3	33 8± 9	0 2>p>0 1	33 3±2 4	28 8±2 4
16 1	1 3±0 4	2.6±0 9	<0 001	6±0 8	2 0±1 0
18 0	13 9±1 4	13 1±1 3		13 3±0 9	15 4±2 0
18 1	13 2±1 5	16 8±2 1	<0 001	17 1±2 3	14 6±1 3
18 ω6	25 8±2 6	18 0±2 3	<0 001	16 4±4 4	22 7±3 4
18 3ω3	0 7±0	0 9±0 3		1 0±0 3	0 7±0 1
0 2ω6	0 3±0 1	0 2±0 1		0 ±0 1	0 3±0 1
0 3ω6	1 8±0 4	2 1±0 6		2 0±0 4	2 5±0 6
0 4ω6	6 8±1 5	6 6±1 6	0 8>p>0 7	7 4±0 8	7 0±1 7
0 5ω3	1 2±0 4	1 7±0 8		1±0 5	1 4±0 9
2? 5ω3	0 6±0	0 8±0 4		0 9±0 3	0 8±0 4
2? 6ω3	2.9±1 1	3 4±1 6		3 7±1 0	3 8±2 0

p is determined from Student s t test for comparison between the groups of alcoholics (n 14) and controls (n=10)

The difference was however not so large as in the cholesteryl esters before and after treatment It is obvious from these figures that the same principal changes of the fatty acid composition were found for cholesteryl esters and for phos phoglycerides in serum from alcoholics in the acute period and after an adequate diet for 14 days

DISCUSSION

In this first paper on the relation between alco holism and changes in the fatty acid composition of the serum lipids we tried to develop a simple and reliable procedure for the routine testing of these changes It has earlier been shown (2) that the serum cholesteryl esters can be separated ac cording to their unsaturation The fatty acids of the cholesteryl esters were determined by GLC and the results were compared with those ob tained by separation of the cholesteryl esters by chromatography on silica impregnated papers and silica gel thin layer plates The last two chromato graphic methods gave results very similar to hose obtained by GLC (Table III) and thus seem to be suitable as routine methods The pa per chromatographic method is the most sensitive but also the least accurate and is rather time con suming The thin layer chromatographic method is very sensitive and accurate and may be used in routine analyses of serum cholesteryl esters in future studies

The serum lipid levels in the present controls were somewhat higher than those in a previous Goteborg series (20) in which the subjects were younger but lower than those found by Bjorn torp and Malmcrona in Goteborg (5) and by Carlson in Stockholm (7) in older age groups The fatty acid composition of the cholesteryl esters differed somewhat from that found in the earlier study eight years ago (11) in the present study the concentration of linoleic acid was higher and that of oleic acid slightly lower The linoleic acid level was somewhat higher than that reported in most oth r studies (17) Corresponding dif ferences as in the cholesteryl esters were also seen in phosphoglycerides This increase in the con centration of linoleic acid may reflect the changes in the fatty acid composition of Swedish mar garine the ma or source of linoleic acid in Sweden The concentration of linoleic acid in the two commonest brands of Swedish margarine has been increased from 3% to 8% or 15%

The major serum changes in the alcoholics were a significant decrease of linoleic acid and a significant increase of monoenoic acids in chol esteryl esters and phosphoglycerides There was no certain change in the level of serum lipids except that of the total cholesterol which was significantly higher in the alcoholics It is a com monplace that a decrease in the intake of poly unsaturated fat results in an increase of the serum cholesterol concentration The finding of an in creased cholesterol content and a decreased lino-leic acid content in the alcoholics supports the explanation that these changes are due to an in sufficient dietary supply

In experiments on rabbits Moore and Williams (16) found an inverse curvilinear relationship be tween the concentration of palmitoleic acid in the plasma and the linoleic acid content of the diet The increase of monoenoic acids in the alcoholics thus seems to lend further support to the as sumption that the changes in the lipid pattern are due to an insufficient supply of linoleic acid Goodman (10) has reported the same changes in the serum fatty acids in association with the use of a diet low in linoleic acid often combined with low calories diet Similar changes have been found among the poor in underdeveloped countries (18) but also in well nourished multiple sclerosis pa tients (4 15) Warembourg et al (24) found an elevation of serum monoenoic fatty acids and a decrease of linoleic fatty acids in several diseases namely atherosclerosis diabetes mellitus liver cirrhosis obstructive or infectious hepatitis and essential hyperlipemia

It is however also possible that the lowness of the linoleic acid level is caused by a toxic effect of the heavy consumption of alcohol According to Zollner et al (25 26 27) the serum linoleic acid concentration is very low in several liver diseases But such an effect can hardly explain the serum changes in our patients for only two (nos 6 and 11) exhibited signs of a possible liver cirrhosis In all cases the increase of the trans aminases was reversible

In five of the alcoholics a second serum sample was taken after they had been on an adequate diet for 14 days In these samples the concentration of linoleic acid in the serum cholesteryl esters and phosphoglycerides was almost normal Whether or

to what extent changes in the serum cholesteryl esters and phosphoglycerides are due to dietary deficits or to a toxic effect of alcohol can be decided only by investigations in which subjects are given an optimal amount of linoleic acid and a varying amount of alcohol and are observed in an experimental metabolic ward

Linoleic acid is necessary for the biosynthesis of arachidonic acid (20 4ω6) which is the major essential fatty acid in mammalia The concentration of arachidonic acid was not reduced in any of the patients studied in fact it was slightly higher both in the cholesteryl esters and the phosphoglycerides Similar increases have been found in kwashiorkor (18) and in two volunteers on an extremely low fat diet (25) The serum level of arachidonic acid may perhaps be controlled by some homeostatic mechanism which prevents a further fall or which even raises the concentration of arachidonate when the level of the serum linoleic acid is low

ACKNOWLEDGEMENT

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IRON DEFICIENCY IN WOMEN OF FERTILE AGE IN A SWEDISH COMMUNITY

II Efficiency of Several Laboratory Tests to Predict the Response to Iron Supplementation

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Abstract Observations on haemoglobin packed cell volume mean cell haemoglobin concentration serum iron serum iron binding capacity and serum iron saturation were made before and after three months of supplementation of iron and placebo to respectively 121 and 63 apparently healthy women of fertile age

A significant change in the observed parameters was defined on the basis of the changes in the placebo group Such a change in one or several of the parameters was observed in about 30 % of the women receiving iron

The property of the observed changes to be predicted from initial values was investigated and it was found that a change in haemoglobin or equally well in packed cell volume was the most predictable change

The best predictor for the change in haemoglobin or packed cell volume was found to be the initial value of the parameter itself A slightly better prediction was obtained by a linear combination of the initial values of haemoglobin packed cell volume and serum iron saturation

The diagnosis of mild forms of iron deficiency and iron deficiency anaemia is still a matter of considerable dispute The problem is of importance since there are suggestions (3) that the prevalence of this disorder is quite high

Generally used criteria include haemoglobin concentration (Hb) packed cell volume (PCV) mean corpuscular haemoglobin concentration (MCHC) serum iron concentration (SeFe) total serum iron binding capacity (TIBC) transferrin iron saturation (SAT) and estimation of the bone marrow iron content or the occurrence of sideroblasts There are no universally accepted limits for any criterion below or above which iron deficiency or iron deficiency anaemia is said to exist

The diagnostic precision of any one parameter can be evaluated only if a master criterion is accepted A criterion based upon the individual s feelings of well being and capacity to perform pertinent social functions would theoretically be the most relevant However such a criterion has not yet been worked out

In the absence of criteria of morbidity there is no satisfactory way to evaluate the diagnostic precision of the different laboratory estimations However if it is accepted that mild forms of iron deficiency and iron deficiency anaemia do exist and that these forms benefit by iron supplementation the problem can be reformulated in the following questions

1) which of the several laboratory tests change most consistently during iron administration to a group of women in which iron deficiency or iron deficiency anaemia can be assumed to be present in at least some cases

2) which of the changes in the different tests can be most accurately predicted by initial values

3) which of the initial values can most accurately predict the change found to be most predictable

The present work was undertaken to find answers to these three questions

MATERIAL AND METHODS

One hundred and eighty four apparently healthy women between 18 and 48 years of age were selected from women attending the General Health Survey of the County of Uppsala The selection was made so as to give an approximately normal distribution of PCV values

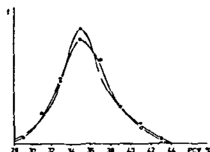


Fig 2 Distribution of initial PCV values in women undergoing a therapeutic trial with placebo ($n=63$) O and iron ($n=121$) ●

On the basis of the changes, regarded as significant, the subjects receiving iron supplementation were classified in two groups responding and non responding individuals The classification was made with respect to each of the six parameter changes studied Thus for each of the six parameter changes a distribution of initial values for each class was obtained Out of the 36 pairs of distributions, only one pair is shown here the distribution of initial values of PCV when the classification was made with respect to change in PCV (Fig. 3)

In each of the 36 pairs of distributions the efficiency of prediction of response on the basis of initial values was calculated as follows for each initial value say the initial value of 36% in Fig 3 the number of responding subjects above that value 6 in Fig 3 corresponds to the number of individuals wrongly classified as non responding when the limit is fixed at 36% i e false negatives The percentage of this figure 6 out of 52 = 12% is conveniently called error of type I Similarly the number of non responding subjects below the value of 36% 31 in Fig 3 corresponds

Table III *Difference of charge in several laboratory parameters after 3 months of treatment with placebo ($n=63$) and iron ($n=121$)*

	Difference (and s.E.) of change		Difference of change
Parameter	Iron –	Placebo	(s.E. of difference)
Hb g%	− 1 4?	0 16	8 9
PCV %	− 3.39	0 41	8.3
MCHC %	− 0 91	0.–1	4.3
SeFe μg	−41 6	6 2	6 7
TIBC μg	−19 7	9.2	–1
SAT	−11 2	1 7	6 6

Table IV *Changes in several laboratory parameters regarded as significant and the corresponding requirements for the choice*

		Fraction of subjects with response	
Parameter	Change	Placebo	Iron
Hb g	− 1.20	2 63	52 1 6
PCV	− 3 60	– 63	5– 1 6
MCHC	− 0.90	5 63	50 176
SeFe μg*	− 60	3 63	5– 176
TIBC μg%	− 34	10 63	5– 176
SAT	− 15 2	– 63	53 176

to the number of subjects wrongly classified as responding when the limit is fixed at 36% i e false positives The percentage of this figure 31 out of 124 = 25% is called the error of type II Thus for each limit of the initial distribution we obtain values for the two errors of classification They may conveniently be plotted on the same graph paper for all the different initial values used as predictors (cp Figs. 4–6)

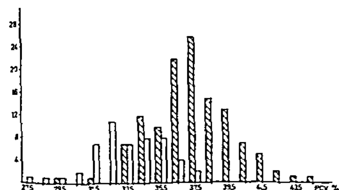


Fig 3 Distribution of initial values of PCV in 52 subjects (open bars) showing a positive response (increase in PCV ≥ 3 60%) and in 124 subjects (cross-hatched bars) showing no response to iron supplementation.

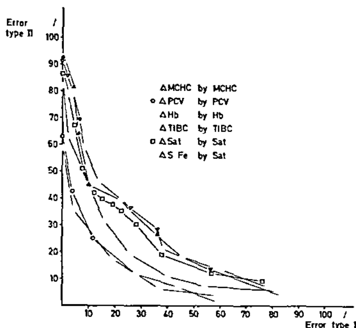


Fig 4 Errors of type I and II for all the six parameter changes when predicted by their respectively best predictors

Fig 4 shows the result of the analysis for those parameters the changes of which were most pre dictable i e these which showed the lowest errors of misclassification It is evident that the best predicted classifications are those based on changes in PCV and Hb

Figs 5 and 6 show the efficiency of prediction of all the six initial values with regard to changes in PCV and Hb respectively

Several different linear combinations of the six initial values were investigated by the method of Kendall (2) for their ability to predict the changes in PCV and Hb The results showed that the changes in PCV and Hb were predicted with the same efficiency When the initial values of MCHC SeFe and TIBC were omitted no reduction in efficiency was noted Therefore only the results for the parameter

$$z = a \quad \text{PCV} + b \quad \text{Hb} + c \quad \text{SAT}$$

predicting the change in PCV will be given here Table V shows the results of the prediction and

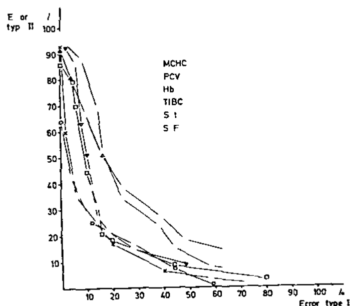


Fig 5 Errors of type I and II when the chance in PCV is predicted by the six different initial values

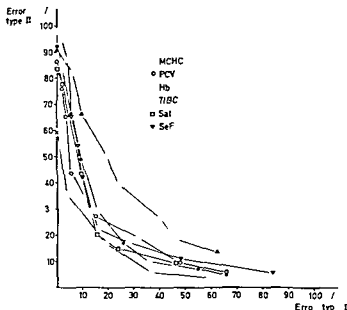


Fig 6 Errors of type I and II when the change in Hb is predicted by the six different initial values

the values of the constants *a* *b* and *c* It is evident that this combination gives a slightly better pre diction than that obtained by using the initial value of PCV as the sole predictor

DISCUSSION

All the six laboratory parameters investigated here showed a significant change during supplementa tion with iron As judged by the distribution of initial values and by the fact that about 30° of the subjects showed a significant change in the parameters in comparison with the subjects on placebo the women investigated here can be ac cepted as a fair representation of a population sample with many cases of mild iron deficiency

In the absence of data related to changes in clinical symptoms there is no conventional way in which to make a choice between the different changes as a measure or criterion of iron de ficiency However the situation in clinical prac tice requires that in addition to an assumed rela tion to clinical symptoms the change obtained during iron therapy should be predictable on the basis of initial values This requirement together with the data *presented in this work* allows the conclusion that changes in Hb or PCV are prefer able as best criteria for iron deficiency

The change consequent on iron therapy in Hb or PCV is best predicted by the initial value of the parameter itself A slight but probably sig nificant improvement in the prediction efficiency is obtained by using a linear combination of th initial values of Hb PCV and SAT

Table V *Errors of type I (false negatives) and type II (false positives) when the outcome of the increase in PCV during iron supplementation is predicted by the following parameter* $=1.7$ *PCV* $+5.4$ *Hb* -0.55 *SAT*

Limiting value of below which a significant increase in PCV is predicted	Error type I	Error type II
145	5	30
140	10	15
135	15	13
130	0	10
125	40	5

ACKNOWLEDGEMENTS

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IRON DEFICIENCY IN WOMEN OF FERTILE AGE IN A SWEDISH COMMUNITY

III Estimation of Prevalence based on Response to Iron Supplementation

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Abstract A new method for estimating the prevalence of iron deficiency anaemia is proposed The method is based on determination of the percentage of subjects showing a significant response in haematocrit or haemoglobin value following supplementation with iron

The method was applied in a study of apparently healthy women in Uppsala The response to placebo and iron supplementation was estimated in a total of 414 trial The percentage of subjects showing a significant response to iron was calculated in each haematocrit interval and the total percentage of subjects judged, on this basis to be iron deficient in a randomly chosen sample from the community was estimated to be 17

Estimations of prevalence of iron deficiency anaemia are generally based upon distributions of parameters that are supposed to be connected with the disease entity and of limits in these distributions below which the disease is considered to be present While community studies of distributions of several iron deficiency parameters are becoming increasingly available there is no general agreement on how to set limits for classification The WHO recommendations (6) for lower limits of haemoglobin concentration (Hb) packed red cell volume (PCV) and mean corpuscular haemoglobin concentration (MCHC) have been shown to be internally inconsistent when applied to women of fertile age in Ireland and Sweden (1 4)

By most definitions *iron deficiency anaemia* is a condition in which the individual s haemoglobin concentration value is below normal due to lack of iron The large variation in Hb values between apparently healthy subjects however makes any such definition rather unsatisfactory (3) But if the definition is modified to a statement about the haemoglobin values of individual subjects in relation to the optimal values of the same individuals it becomes much more reasonable A criterion of iron deficiency anaemia of this kind could be based upon the response of the individual s haemoglobin concentration to iron supplementation on the assumption that such a response if it occurs brings the haemoglobin level of that subject to his optimal value

Iron deficiency may be defined in terms other than those related to the concentration of red cells or haemoglobin For example the serum iron concentration (SeFe) or the total iron binding capacity (TIBC) or the transferrin saturation (SAT) may be more related to the clinical condition However as discussed in detail by Garby et al (5) the absence of quantitative relations between the laboratory parameters and clinical symptoms in mild cases of iron deficiency (2) makes it impossible to decide in favour of one laboratory parameter rather than another If in spite of this one assumes that mild forms of iron deficiency do exist and that iron therapy will in fact alleviate hidden symptoms the problem of recognizing such individuals arises In a previous study (5) it was shown 1 that iron supplementation to apparently healthy women of fertile age *with slightly lower than average* values of their PCV resulted in a response in Hb and PCV that was more reproducible than the response in MCHC SeFe TIBC and SAT 2 that the response in Hb and PCV was more accurately predicted than the response in any of the other parameters examined and 3 that the response in Hb

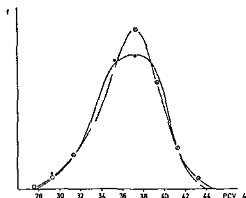


Fig 1 Distribution of PCV values before treatment O = subjects subsequently on placebo ● = subjects subsequently on iron

and PCV was more accurately predicted by the initial values of either of the two parameters than by the initial values of any of the remaining parameters Therefore in a group of women containing iron deficient subjects the largest number showing a response to iron supplementation will be found by using Hb or PCV as response parameters and the best predictors for response will the initial values of Hb or PCV

In the present work we report on an attempt to estimate the prevalence of iron deficiency anaemia among women of fertile age in Uppsala The estimate is based on a determination of the percentage of women who showed a response in their PCV value following supplementation with iron which was equal to or greater than a response obtained by random factors as evaluated by studies in subjects on placebo tablets

MATERIAL AND METHODS

Apparently healthy female individuals of fertile age attending the County General Health Survey were invited to take part in the study They were selected on the basis of their PCV value so that the total material would have a slightly lower mean PCV value than the figure obtained in a random sample The distribution of PCV values in the material is shown in Fig 1 A brief medical history was recorded and an examination made in order to exclude individuals with diseases influencing the test relevant to this study

The subjects were told that their blood value was borderline low and that therapy with iron would most probably be of benefit A total of 359 women took part in the study Of these 110 women were given placebo tablets containing 150 mg of lactose and told to take one tablet every day for three months The remaining 249 women received iron tablets containing 60 mg of elemental iron as ferrous fumarate and were told to take one tablet daily for three months Of these latter subjects 55 were reinvestigated after another three months of iron supplementation.

Packed red cell volume determinations were performed at the beginning and the end of the study period In 47 of the women receiving placebo and in 116 of those receiving iron PCV determinations were performed on finger prick blood on both occasions In the remaining subjects the determination was performed on both occasions on blood drawn without stasis from a cubital vein The blood samples were taken with subjects sitting or lying horizontally for less than 5 min Before sampling, the subjects had been sitting for at least 15 min The method of determination was carried out as described previously (4)

RESULTS

The subjects given placebo had a mean initial PCV of 36 64% with a standard deviation of 2 96% The mean change in PCV ΔPCV after treatment with placebo was −0 28% with a standard deviation of 2 09% (S E = 0 20%) The distribution of ΔPCV is shown in Fig 2 The linear regression of ΔPCV on the initial value was

$$\Delta PCV = 5\ 23 - 0\ 151 \quad PCV$$

with a standard error of the regression coefficient of 0 068 (see Fig 3)

The subjects receiving iron supplementation had a mean initial PCV value of 36 72% with a standard deviation of 2 88% The change in PCV after treatment is shown in Fig 3

The change in PCV in the 55 women who took part in a second identical trial with iron was

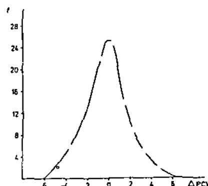


Fig 2 Distribution of the change in PCV ΔPCV in 110 subjects receiving placebo supplementation for three months

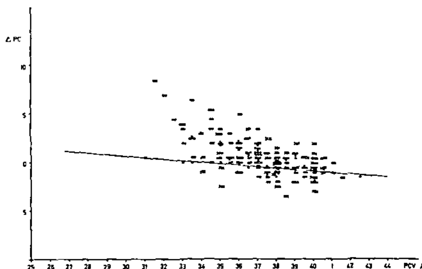


Fig 3 Relation between the initial PCV value and the change in PCV after three months of treatment with placebo ($n=110$) × and iron ($n=304$) ● The regression line for the placebo subjects is also shown.

+0 34 (S E = 0 21 %) during the second pe riod

The calculation of the percentage of subjects showing a significant increase in PCV during iron supplementation was made in the following way Among the subjects who received supplementa tion with iron a certain fraction showed no response i e they responded to the iron supple mentation in the same way as did the control subjects to placebo Since the latter subjects showed a symmetric response around the mean value of − 0 28 ⁄ (cp Fig 2) it can be assumed that those who did not respond to iron supple mentation also showed a symmetric response around the same mean value The number of non responding subjects on iron supplementation was therefore calculated in each PCV interval by multiplying by two the number of subjects fal ling below the regression line of the controls The number of responding individuals was then easily found

The result of the calculations is shown in Fig 4 This figure also shows the distribution of PCV values found in 5683 women of fertile age in a random sample from the community (4)

The percentage of responding subjects in the community was calculated by correcting the pre valence figure in each PCV interval for the rel ative frequency of individuals in that interval in the community (Table I) It was found to be 17 % A 95 confidence limit of this figure was estimated by assuming that the variate in each PCV interval was binomially distributed it was found to be 10–24 %

The distribution of initial PCV values among responding and non responding subjects was cal culated from the distribution found in the random sample (cp Fig 4) by subtracting the relative number of responding subjects found in the pres

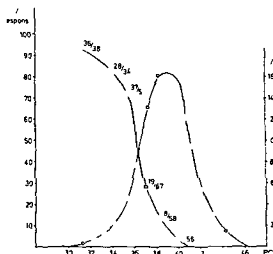


Fig 4 Percentage response to iron supplementation in 304 trials in relation to the initial PCV value ☐ The numbers at each point represent the subjects showing a response and the total number of subjects in the interval of PCV This figure also shows the relative prevalence of PCV values in unit PCV interval in a randomly selected sample ○ (4)

Table I *Percentage of subjects responding to iron supplementation at different levels of PCV and their respective prevalence in the community of Uppsala*

PCV	Per cent of subjects in unit PCV interval in the community sample[a]	Per cent of subjects who respond to Fe[b]	Per cent of total population who respond
31 0	0 25	93	0 2
32 0	0 6	90	0 5
33 0	1 1	87	1 0
34 0	2 0	82	1 6
35 0	3 5	76	2 6
36 0	6 6	58	3 8
37 0	10 6	28	3 0
38 0	15 0	17	2 6
39 0	16 4	9	1 5
40 0	16 0	3	0 5
		Per cent total response	17 3

[a] From Garby et al (4)
[b] From the present study

ent study The result is shown in Fig 5 The discrimination between responding and non responding subjects on the basis of initial PCV values is illustrated in Fig 6 In this figure an error of type I denotes the percentage of wrongly classified responding subjects i e false negatives and an error of type II denotes the percentage of wrongly classified non responding subjects i e false positives Table II shows the two types of error as a function of different levels of PCV

DISCUSSION

Previous estimates of the prevalence of iron deficiency anaemia have been based upon determination of the relative number of randomly selected subjects having haemoglobin levels below a certain arbitrarily chosen limit usually 11 5 or 12 0 g% and on the assumption that the great majority of such subjects are anaemic because of lack of iron In addition to the introduction of arbitrary limits the method suffers from the disadvantage that widely different estimates will be obtained depending on the chosen limit This is due to the fact that the distribution of haemoglobin or PCV values at least in so called developed countries is quite steep around the values of interest For example in the community of Uppsala the percentage of apparently healthy women showing haemoglobin values below 11 5 and 12 0 g% (PCV values below 36 0 and 37 5 %) was 15 and 30% respectively (4)

Several studies have been made in which iron supplementation has been given to apparently healthy male and female subjects The previous work has been reviewed in detail by Garby et al (4) In these studies however the number of subjects investigated has usually been quite small and the data have not been subjected to analyses of the kind described here

The present study shows that 17 % of women of fertile age in the community of Uppsala display a significant response in their PCV value to supplementation with iron Although it is not possible

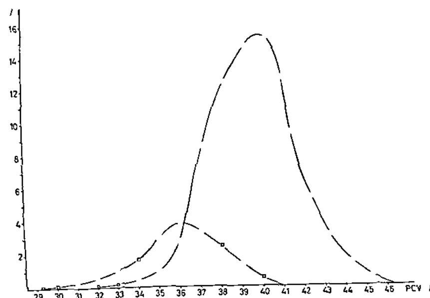


Fig 5 The distribution of PCV values in subjects showing a response □ and in subjects showing no response O to iron supplementation

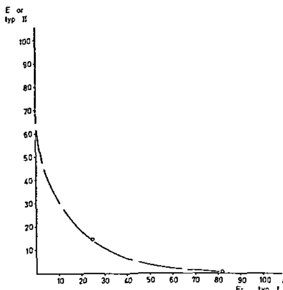


Fig 6 Discrimination of non responding and responding subjects on the basis of initial PCV values An error of type I denotes the percentage of wrongly classified responding subjects and an error of type II the percentage of wrongly classified non responding subjects

to prove by the results of the present study that an increase in PCV value of this kind is of any benefit to these persons it seems reasonable at present to accept the criterion used here as much less arbitrary than a criterion based on a limit for haemoglobin or PCV values If the figure is taken to signify prevalence of iron deficiency anaemia it shows that this disease is quite common in the community

The distribution of PCV values among responding and non responding subjects shows a considerable overlapping (Fig 5) This fact probably reflects the considerable variation in optimal blood values among healthy individuals The data obtained here (Figs 5 and 6 and Table II) provide a basis for a strategy of supplementation of iron to individuals in a community which is screened by Hb or PCV determinations If a figure of 10 is accepted for making an error of type I (wrongly classifying an individual as being healthy) about 30 % of healthy persons will be classified as being iron deficient this strategy implies that a limit of 38 5 must be used If instead one accepts an error of type I of 20 the corresponding type II error is about 17 and the limit will be 37–37 5° PCV

Table II *Errors of type I and type II for different limits of PCV*

Limiting value of PCV below which a response is predicted	Error type I ( )	Error type II ( )
39 0	37	6
38 0	21	17
37 0	10	32
36 0	3	53

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CASE OF TAKAYASU S SYNDROME ACCELERATED (INITIATED?) BY ORAL CONTRACEPTIVES

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Abstract A case of Takayasu s syndrome in a woman, aged 30 is described The onset of the syndrome cor responded in time with the administration of oral contra ceptives Since arterial occlusions were registered as well it cannot be excluded that the oral contraceptives contri buted at least to the acceleration of the arterial illness

Takayasu s disease represents an arteritis which is usually ascribed to auto-immunity and belongs to the collagenoses It is as a rule localized to the arch of the aorta and to the big arteries taking off from its convexity It may extend in the proximal direction so as to involve the coronary arteries and in the distal direction to involve the thoracic part of the aorta, usually ending at the level of the diaphragm Occasionally a more ab dominal localization may be encountered usually around the level of the renal arteries (for refer ences see (1) and (4)) Thrombosis of the involved arteries for instance the subclavian or the carotid artery may add to the decrease in arterial supply of the involved regions

OBSERVATION

The following case may serve as an illustration of the entangled relationships which may be en countered

Woman, born 18 May 1935 Both parents had gall blad der disease her father also rheumatoid symptoms 20 years previously She has one brother three years her junior and this brother suffers from bronchial asthma. She had to leave school after four years becaus of se ere headaches part cularly when looking on snow in sunshine These headaches had started when she was only a very young g rl Her menarche dated from the age of 11 when she also had rheumatic pains" in wrists and ankle joints During the d ys preceding and also dur ing the first days of each period she suffered from se ere headaches, vomiting and even syncope (on the first day of the period) She married in 1955 and has had two pregnancies (a girl in 1958 breach position, and a boy in 1963 head position) She was unbel e ably healthy" during the pregnancies and her premenstrual difficulties were considerably reduced. Five years before her first admission she took part in building the family s house and did so with pleasure and energy The last two years, however she has not felt up to par and readily becomes fatigued In the fall of 1964 she had some kind of vesicular stomatitis in connection with her periods and was advised to take oral contraceptives. She started this medication on the Christmas Eve of 1964 and continued until Oct 1965 In the middle of Jan 1965 at a health examination she was found to have a considerably in creased sedimentation rate of her red blood cells (87 in 1 hour) This persisted and was not rel eved by a chole cystectomy in March 1966 On two occasions during 1966 the lower half of the visual field disappeared for some few minutes, whilst in a sitting position In the fall of 1966 she felt a peculiar throbbing in her head She was admitted to the clinic on Dec 14 1966 and kept under observation until Dec 23

On admission her general condition was fair She was slender hands and feet cold Temperature fairly normal pulse rate 70–100 ESR 92, eyegrounds normal B.P right arm 110/60 left arm not measurable right leg 160/60 left leg 160 65 Heart size normal (3 0 ml/m²) A systolic murmur all over the *precordium maximal in the* 2nd right intercostal space and extending to the carotid arteries (right grade 5 left grade 3–4) Pulses reduced in the rad al internal carot d and temporal arteries of the left side Oscillometry reduced excursions of left arm Femoral arteries audible pulsations ECG reduced am plitude of T waves Fibrinogen 0 87 albumin less than 4.5% gammaglobulin abo e 1 4 %

Aortography as usual by means of a Seldinger cath eter introduced through the femoral artery re ealed the following alterations

1 Moderately reduced caliber of innominate artery
2 Caliber of left carotid artery reduced to for some 4 cm from its take-off from the aortic arch
3 The left subclavian artery d d not fill
4 Contrast may have been passing into the left sub-clavian from the left vertebral artery (subcla ian steal")
5 Abdominal aorta and renal arteries normal.

She was treated with prednisolone and kept under observation in the clinic also from Jan 11 to 19 1967 when she obviously was considerably improved the ESR being reduced to 18 mm/1 hour

COMMENT

In this case the diagnosis Takayasu s syndrome seems to be well substantiated

Her general fatigue during the last two years preceding her admission to our clinic seems reasonably to be ascribed to her arterial affliction However the onset of her general symptoms also seems to coincide with the administration of oral contraceptives There is accordingly reason to consider whether this medication has had anything to do with her illness On the one hand the presence of L E cells has been described in connection with the administration of oral contraceptives (7 8) and although no L E cells were looked for in the present case we usually regard the Takayasu s syndrome as a collagenosis thus genetically related to the L E It would accordingly not be at all unreasonable to suppose that administration of oral contraceptives in this may have pulled the trigger

On the other hand only three weeks elapsed between the onset of this administration and the observation of the remarkably increased sedimentation rate of the red blood cells admittedly rather a brief period

However although the correspondence in time between the onset of her symptoms and the administration of oral contraceptives seems obvious it does not appear warranted to presume a causal relationship Nevertheless even if the arterial diseases had been present before the patient received her oral contraceptives which of course is a mere assumption we must remember that arterial thrombosis may be favoured by oral contraceptives and hence an acceleration of the arterial illness may easily be speeded up by such agents

There is evidence suggesting particular precaution in the use of oral contraceptives in women with migraine In this case there was a solid history of migraine and the throbbing discomfort in her head in connection with the disease should be emphasized

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YERSINIA ENTEROCOLITICA INFECTION ASSOCIATED WITH BRUCELLA AGGLUTININS

Clinical Features of 24 Patients

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Abstract The clinical pictures of 24 patients having high agglutinin titres against both Brucella abortus and Yersinia enterocolitica type IX are described Clinical serological and epidemiological evidence is presented suggesting that the causative agent is Yersinia enterocolitica type IX in the majority if not in all instances Fever diarrhoea and abdominal pain were the dominant symptoms However the series includes some atypical cases one with Reiter s syndrome one with signs resembling collagen diseases, but which could have been a septicaemic form of Yersinia enterocolitica infection and one with recurrent fever and muscular pain resembling brucellosis or polymyalgia rheumatica

In the past few years it has become evident that human infections due to Yersinia enterocolitica (formerly Pasteurella X) are not rare at least in some countries (11 13 19 21) This bacterium first isolated by Hassig et al in 1949 (8) may give rise to clinical pictures similar to those caused by Yersinia pseudotuberculosis (syn Pasteurella pseudotuberculosis) mesenteric lymphadenitis enteritis erythema nodosum and rarely septicaemia The 23 cases described in the literature up to 1966 including those caused by Bacterium enterocoliticum were reviewed by Mollaret (11) Since that time many cases have been observed especially in Sweden (13 21) The origin of the infection in man is not as yet known In animals Y enterocolitica has been isolated especially from chinchillas (3)

Based on 0-antigens Winblad (20) divided 105 strains of Y enterocolitica into eight serotypes The type with 0 antigen III was found to be the most common. The taxonomic position of Bacterium enterocoliticum (16) is not clear Frederiksen (6) was the first to suggest that it should be included in the species Y enterocolitica

Recently a marked cross agglutination between Brucellae and a subtype of Y enterocolitica referred to as type IX was observed in a series of 24 patients having high agglutinin titres against both Brucella abortus and this type of Y enterocolitica (1 2) Absorption of the sera with Y enterocolitica type IX removed brucella agglutinins in every case Absorption of the sera with Brucella abortus did not reduce the yersinia titre significantly in six instances reduced it significantly in twelve and removed all detectable yersinia agglutinins in six The results of the cross absorption tests thus suggested that the infective agent was Y enterocolitica type IX rather than any Brucella in the majority of the cases The purpose of this paper is to analyse the clinical pictures of these patients in order to obtain information to help in determining whether the diseases were more likely caused by yersinia or brucella infection

MATERIAL AND METHODS

The series consists of 24 patients found to have in their sera agglutinins against Yersinia enterocolitica type IX in titres of 160–2560 and brucella agglutinins in titres of 160–2560 (corresponding to 100–1600 I U) An infection due to Yersinia had been suspected in four of the patients. Sixteen patients were found to have brucella agglutinins in specimens sent in for the routine Widal test and four in specimens for toxoplasma antibody studies (2)

The age of the patients varied between 9 and 55 years, the mean being 35 years. Eleven of them were male and

Table I *Symptoms signs and some laboratory data of the 24 patients with positive Brucella agglutination test*

Case no	Sex	Age (y)	Onset of the disease	Fever (d)	Diarrhoea (d)	Abdominal pain (d)	Headache	Backache	Joint swelling	Highest ESR	Highest WBC	Highest aggl titre Y ent type IX	Highest aggl titre *Brucella* abortus	Remarks
1	♂	14	Feb	12	7	+	—	—	—	14	8 000	1280	640	Mesenteric lymphadenitis
2	o	43	Feb	rec	—	—	—	—	+	107	9 600	640	160	*Muscular pain*
3	♀	50	Mar	rec	—	—	+	—	+	57	13 400	320[a]	160	Purpura hepatitis later arthritis
4	♀	29	June	21	14	—	—	—	—	11	5 200	640	160	Erythema nodosum
5	♀	25	June	+	7	—	—	—	—	28	3 700	320	3⌞0	
6	♂	25	June	37	14	14	+	+	+	68	19 100	320	160	Reiter s syndrome
7	♀	51	July	19	3	2	—	—	—	83	4 900	1280	1280	Suspected appendicitis
8	♀	27	Aug	14	—	40	+	—	(+)	69	4 300	1280	640	
9	♀	40	Aug	14	14	14	—	—	—	4	4 600	640	320	
10	♂	23	Aug	7	4	—	+	—	—	50	7 400	1780	640	Signs of meningeal irritation
11	♀	51	Aug	2	14	14	—	—	(+)			320	160	
12	♀	51	Aug	14	21	+	+	+	—	58	6 100	1280	640	
13	♀	55	Aug	14	14	14	+	+	—	88	7 900	640	160	Erythema nodosum acute bronchitis
14	o	42	Aug	20	—	20	+	+	—	70	4 700	160[a]	160	Arthralgia
15	♀	51	Aug	—	25	+	+	+	—			640	320	Sore throat
16	♀	19	Sep	7	4	+	(+)	—	—	56	20 200	640	640	Mesenteric lymphadenitis
17	♂	49	Nov	14	9	14	—	+	—	93		2560	2560	
18	♂	9	Nov	12	7	8	(+)	—	—		14 700	1280	160	Mesenteric lymphadenitis
19	♂	40	Nov	7	—	7	—	—	—	93	16 300	3⌞0	320	Erythema nodosum arthralgia
20	♀	29	Nov	7	7	7	+	+	—	21		2560	320	
21	♂	32	Dec	35	14	14	—	+	+	35	11 000	640	320	
22	♂	35	Dec	14	14	14	+	—	—	29	9 600	1280	640	
23	o	25	Dec	16	—	14	—	—	—	40	4 600	1280	640	Arthralgia
	♀	24	?	—	—	—	—	—	—			320	320	Foetus mortus Abdominal pain 3 months previously

[a] After absorption with Br abortus the titre against Y enterocolitica was <20
rec = recurrent + = the symptom was present (+) = the symptom was present already before the present illness

13 female (Table I) Ten of the patients were from Helsinki the others from different parts of Finland including the level of the Arctic Circle The material was collected during 1967

The data from hospital records were supplemented by a detailed questionnaire which was answered by all patients. Seven of the patients were personally seen by the authors at the follow up examination

In 20 cases the first blood specimen for serological studies was obtained 7–30 days after the onset of symptoms and after one and a half to four months in three others In one case the onset of the disease was difficult to determine In all but one case a second blood specimen was obtained two to nine months after the first

The agglutination and absorption methods are described elsewhere (2) The cross-reacting Yersinia enterocolitica strain MY 79 used in the agglutination test was obtained from Dr Birgitta Nīléhn, General Hospital Malmo Sweden. This type of Y enterocolitica was not included in the antigen scheme of Winblad (70) and according to his suggestion is referred to in this paper as type IX Brucella abortus antigen was prepared from strain 544 obtained from Dr W J Brinley Morgan FAO/WHO Brucellosis Centre Weybridge England

RESULTS

The symptoms signs and some laboratory data of the patients are shown in Table I

The majority of the patients had a combination of fever with abdominal pain and/or diarrhoea The pain was localized in the upper abdomen in nine cases in the right lower quadrant in seven and was more diffuse in the others In four of these cases an acute appendicitis was suspected which led to operation During the surgery acute mesenteric lymphadenitis was verified in three cases

Six patients had a *fever of 39 C or over* and an additional ten patients had a fever ranging from 38 C to 38 9 C Erythema nodosum developed in three patients from seven to nine days after the onset of the gastrointestinal symptoms Twelve patients had headache which was severe in some cases One patient had signs of meningeal

irritation, but the cerebrospinal fluid was normal

In one case (case 6) the clinical picture was compatible with Reiter's syndrome Five other patients reported that they had joint swelling during the acute illness However two of these five patients had had mild polyarthritic symptoms before the present illness These two patients had no signs of synovitis or erosive arthritis at their follow up examination five and eight months after the onset of the present illness respectively Additionally eight patients had backache two complained of arthralgia and one (case 2) had severe muscular pain for four months

Four of the patients complained of dysuria (cases 2 3 6 and 21) In addition to these cases the urinary sediment was examined in 18 others and was normal in every case

In one patient (case 14) the spleen was possibly palpable one patient (case 3) had enlarged liver and three patients (cases 3 6 15) enlarged lymph nodes in the neck. Only one third of the patients had leucocytosis ranging from 9600 to 20 200/mm³ ESR varied from 21 to 107 mm/h in 17 cases and from 4 to 14 in three

Only case 3 was icteric during the present illness S-GOT and S-GPT were determin d in eight cases They were normal except in case 2 (S-GPT 88 Wroblewski units) and in case 3 described below The alkaline phosphatases were normal in seven of these cases and elevated in case 3 Bromsulphalein retention was determined in case 6 (8 7 % /45 min) and in case 14 (12 2 % /45 min)

Three cases are presented in more detail

Case 3

A woman aged 50 fell ill with fever up to 39 8 C itching and headache After five days she developed petechiae and was admitted to hospital The first fever period lasted for 20 days No defects in blood coagulation were found The thrombocyte count was normal. The patient was anaemic the lowest haemoglobin value being 8.5 g/100 ml Except for the purpura no other pathological physical signs were observed. Blood culture was negative The patient received continuous intensive antimicrobial therapy for two months. Despite this she had three further periods of fever up to 39 1 C each lasting about one week

Three months after the onset of the disease the patient became icteric (bilirubin up to 5 2 mg 100 ml) with elevated S-GOT (up to 6_ mIU) and alkaline phosphatases (up to 11.0 Bessey Lowry Units) L.E cells were not found and tests for rheumatoid factor were negative In paper electrophoresis the gammaglobulin was 1.8 g/100 ml The liver was enlarged and she had enlarged lymph nodes in the neck.

Corticosteroid therapy was started and there was a rapid improvement of the patient s condition including the normalization of the bilirubin level and ESR. After three weeks liver biopsy showed only slight parenchymal degeneration Cholecystography was normal. The patient was symptomless for five months. After that she has had swelling and pain on motion in most of her joints The tests for rheumatoid factor remained negative

Three months after the onset of the disease the agglutination titre with Yersinia enterocolitica type IX antigen was 3_0 Eight months later it was less than 20

Case 6

A 25 year-old male patient was admitted to hospital because of abdominal pains, diarrhoea and dysuria, which all lasted for two weeks Fever appeared during the same period (up to 38 8 C) and lasted for five weeks Ten days after the onset of disease the patient experienced eye symptoms due to uveitis Three weeks after the onset of gastro-intestinal symptoms the knees, fingers and wrists became swollen and painful Tests for rheumatoid factor were negative The sacro-iliac joints, as well as peripheral joints, were radiologically normal. During penicillin and corticosteroid therapy the symptoms subsided

Y enterocolitica type IX titres in specimens taken one month and eight months after the first symptoms were 3 0 and <20 respectively

Case 13

A 55 year-old woman fell ill with fever which rose to 40 0 C diarrhoea and diffuse abdominal pains, lasting two weeks At the ninth day several painful nodes typical of erythema nodosum developed on both legs These disappeared after three weeks Y enterocolica type IX titre was 640 two weeks after the onset of the disease and < 0 six months later

In 23 cases out of 24 a second blood specimen for serological studies was obtained from two to nine months after the first. In every case the titre against both Yersinia and Brucella was significantly reduced i.e from two to seven twofold dilution steps In eight specimens there were no detectable agglutinins against either bacteria In the other 15 the diminution of agglutinin titres against both Brucella and Y enterocolitica type IX was quite parallel The titres against Y enterocolitica type IX in these 15 specimens were 20–160 In ten of these the brucella agglutinin titres ranged from 20 to 160 and in five sera the titre was less than 20

Case 3 described above along with cases 5 11 12 14 and 15 had no detectable yersinia antibodies in their sera after absorption with Brucella abortus As seen in the table the dominant

symptoms in these patients were fever diarrhoea and abdominal pains except in case 3

In one case of mesenteric lymphadenitis an attempt was made to isolate Yersinia with negative result

DISCUSSION

It is well known that the symptoms of human brucellosis vary very much (4 17) On clinical grounds alone it is difficult to argue with certainty that the presented cases could be separated from the broad spectrum of clinical pictures included in human brucellosis However there are certain data in favour of infection with Yersinia enterocolitica type IX in this series Firstly bovine brucellosis has been totally eradicated in Finland (9) and bacteriologically verified animal brucellosis has not been encountered in Finland for more than ten years (18) Secondly the few human cases in the past few years diagnosed as having brucellosis based on positive agglutination tests have not led to the finding of any case of animal brucellosis in the environment of these persons Thirdly the agglutinin absorption tests 2) suggest that at least in 18 of the 24 patients the infection is more likely to have been caused by Y enterocolitica type IX than by any Brucella In six cases the results of the cross absorption tests were inconclusive

However the finding of high agglutinin titre against a bacterium does not imply that the patient s disease is causally related to it This is especially true in our series since it was not *possible for practical reasons to show a rise in* the agglutinin titres or to isolate the infective agent Theoretically moreover the infection in the present series might have been caused by another microbe which shares an antigen with both Yersinia and Brucella

In the majority of the cases presented the clinical picture corresponds to the earlier descriptions of Y enterocolitica infection (11 13 21) Even in four of the six cases with no detectable yersinia agglutinins after absorption with Brucella abortus the picture was compatible with yersinia infection Most patients had presented to a physician or been admitted to hospital because of gastrointestinal symptoms often accompanied by fever In most cases the data of the other symptoms (headache backache) were obtained through a later questionnaire and were not always mentioned in the hospital records

However our series presents some exceptions to the general clinical pattern (cases 2 3 6 14 and 24) Case 2 with recurrent fever and severe muscular pains of four months duration could on clinical grounds have been a case of brucellosis *or polymyalgia rheumatica seu arteritica.* The combination of male sex and rather young age (43 years) contradicts the last possibility

The course of the disease in case 3 suggests the possibility of some collagen disease However it resembles somewhat the two septicaemic cases of Yersinia enterocolitica infection described by Hassig et al (8) Brucellosis could be a third alternative

Case 6 had a clinical picture compatible with Reiter s syndrome The aetiology of Reiter s syndrome is not known in most cases (5) It has been shown that complete or incomplete Reiter s syndrome may follow Shigella infection (14) Bedsonia and mycoplasma organisms have been isolated from the synovial fluid in some cases of Reiter s syndrome (5 15) One case of Reiter s syndrome in our rather small series could of course have been caused by chance alone However this raises the question of a possible causal relationship between Yersinia enterocolitica type IX infection and Reiter s syndrome

One patient with foetus mortus (case 24) had had abdominal pains three months previously which could have been caused by Y enterocolitica infection Case 14 with fever backache abdominal pain and palpable spleen could have *been considered also a case of brucellosis on* clinical grounds Additionally in this case the result of cross absorption was inconclusive

Y enterocolitica type IX has been considered to be rather rare until now Nilehn in Sweden has isolated this type of Y enterocolitica three times from stools of patients with gastrointestinal symptoms (12) One strain of Y enterocolitica isolated from a mesenteric lymph node by Jansson in Finland in 1965 (10) was later found to belong to type IX Of ten cases of erythema nodosum caused by Y *enterocolitica five* were due to type IX (7) Two of these cases are included in the present series

In conclusion the clinical serological and epidemiological evidence presented above seem to indicate Yersinia enterocolitica type IX as the

causative agent of the disease at least in most of the cases presented According to this series of 24 patients Y enterocolitica type IX is not rare in Finland

Addend m After the submission of the manuscript we have i olated nine strains of Yersinia enterocolitica type IX from stools of patients with erythema nodosum and/or gastrointestinal symptoms Eight of these patients showed high agglutinin titres against both Yersinia enterocolitica type IX and Brucella abortus

In a subsequent study several cases of acute and sub acute arthritis were observed which were probably as sociated with Yers n a enterocolitica infection (P Ahvonen K Sievers & K Aho Arthritis associated with Yersinia enterocolitica infection To be publ shed)

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PLATELET ADHESION A COMPARISON OF FOUR METHODS

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Abst act A direct comparison of Wrights, Hellems whole blood and platelet rich plasma/adenosine diphosphate and Salzmans methods for platelet adhesion has shown good correlation between Hellems and Salzmans whole blood methods ($P < 0\ 01$) a weaker yet significant correlation between Wrights and Salzmans methods ($P < 0\ 05$) but no correlat on between any of the three whole-blood methods employed and Hellems PRP/ADP method

Since the description by Wright in 1941 (24) of a method for estimating platelet adhesiveness a number of additional in vitro methods have been devised for the same purpose (6 9 10 12 14 16 18 21)

The use of the sam• method has provided con tradictory results in the hand of different workers Ashby et al (1) using Wrights method found an increase in platelet adhesiveness after smoking but this could not be confirmed by Murchison and Fyfe (17) who used the same method Further more using different methods of estimating plate let adhesiveness in similar groups of patients contradictory results have been obtained McDonald and Edgill (14) could demonstrate in creased platelet adhesiveness in patients with ischaemic heart disease using their own modifica tion of Wrights test In contrast using a similar group of patients but applying Hellems plate let rich plasma method Rozenberg and Stor morken (20) were unable to find any evidence of increased platelet adhesiveness In a group of patients with diabetes mellitus Ödegaard et al (25) using Hellems whole blood technique found evidence of decreased platelet adhesiveness whereas Bridges et al (3) applying Wrights method found platelet adhesiveness to be in creased

For these reasons a comparison has been made of four commonly used methods of estimating platelet adhesiveness namely Wrights method as modified by McDonald and Edgill (15) Hellems whole blood method (9) Hellems plasma method (10 22 23 26) and Salzmans method (21) There are several important differences between these methods (Table I) and a direct comparison has been made between each method and the other three

METHODS

The experimental details of the four method used were as follows.

Modified Wright rotator test

Within 5 min of venepuncture two ml citrated blood are transferred into a conical pyrex flask fixed horizontally into a drum rotating at 3 rpm. After 20 m n a sample for platelet count is taken from the flask.

Hellems whole blood method

Fifteen min after venepuncture citrated whole-blood is forced through a glass-bead filter at a constant rate by a motor-driven syringe The filter is made of polyvinyl tubing with an internal diameter of 5 mm and contains 5 g Ballotini 8 glass beads (Glas Export AG Liberec Czechoslovakia) The collection t m for 1 ml was 23.5 sec giving a platelet contact time of ?8 ? sec

Hellems platelet rich plasma adenosine diphosphate method

Platelet rich plasma (PRP) was prepared by centrifuging citrated blood at 200 g for 15 min in a Wifug centrifuge at room temperature 30 min after enepuncture Adenosine diphosphate (ADP) from a freshly prepared solution (pH 7 4) to gi e a final concentration of 0 1 μg ADP ml PRP was then added The PRP ADP mixture was then immediately passed through the same glass-bead filter and in the same manner as in Hellems whole blood method.

Table I *Salient features of the four methods used in this study*

Method	Wright	Salzman	Hellem	Hellem PRP/ADP
Blood specimen	Citrated blood	Native blood	Citrated blood	Citrated PRP/ADP
Temperature	20 C	37 C	20 C	20 C
Delay[a]	5	0	15	45
Driving force	3 rpm	Vacuum	Motor driven syringe	Motor-driven syringe
Contact surface	Glass flask	Glass beads 0 4 mm diam	Glass beads 0 5 mm diam	Glass beads 0 5 mm diam
Delivery time	—	35	23 5	23 5
Contact time	20	Mean 3 5	28 2	28 2

[a] Interval between venepuncture and process for adhesion

Salzman s method

Venous blood is drawn directly through a glass bead filter made of polyvinyl tubing, internal diameter 4 mm, containing 2.5 g Ballotini 9 beads (Glas Export AG Liberec Czechoslovakia) by a 7 ml vacutainer (Becton, Dickison & Co Rutherford, N J USA) containing Na_2EDTA A collection time of 35 sec was used giving a mean contact time of 3 5 sec

Platelet adhesion as determined from duplicate estimations was expressed according to the formula

$$\frac{\text{initial count—final count}}{\text{initial count}} \times 100$$

Platelet counts in Wright s method were performed by he method of Bjorkman (2) In the other three methods counts were obtained by the use of an electronic particle counter (Celloskop 101 AB Ljungberg, Sweden) Prior to counting platelets in whole blood with the particle counter the erythrocytes were centrifuged off Throughout the investigations the counter was repeatedly checked against microscopic counts The usefulness of particle counters in platelet adhesion studies has been shown by Stormorken et al. (23)

In experiments involving citrated blood this was prepared by adding 9 volumes of blood to one volume of 3 13 g trisodiumcitrate dihydrate after which the test tube was inverted 10 times

S liconised equipment (Siliclad Clay Adams Inc USA) was used in all experiments with the exception of the surfa es exposed to the platelets for adhesion

ADP (Sigma Chem Co USA) was obtained from a deep frozen THAM buffered (tri hydroxymethylamino methane) saline solution containing 400 μg ADP ml (pH 6 8)

METHODS

Subjects

Blood for the tests was taken from patients and healthy controls taking part in a study of platelet adhesion in ischaemic heart disease (to be published) Fifty were healthy controls and 50 ambulant out patients who had previously sustained a myocardial infarction at least 2 months prior to this investigation Blood was taken after an overnight fast The subjects did not smoke during the period of fast After resting for at least 30 min venous blood was taken for estimations of platelet adhesion and haematocrit values Only subjects with normal haematocrit values men 40–54 and women 36–47 were included in this investigation For each comparison two different tests were performed on 25 subjects

Additionally the normal range of values for each of the methods employed was obtained from 50 healthy subjects of both sexes free from symptoms suggestive of chronic or acute illness aged 20–70 years

Statistical methods

The methodological error in each method was obtained using the blood of 20 subjects Duplicate estimations were twice performed within 1 hour of each other and processed separately The methodological error wa determined from the formula $\sqrt{d^2/2n}$ where d was the difference between paired estimations

The degree of correlation between the four methods used was determined by Wilcoxon s rank correlation method

RESULTS

The methodological results obtained from determinations of platelet adhesion using all four methods employed in this study are shown in Table II

The results of the direct comparisons between the methods are shown in Figs 1–6

Whole blood methods

When Hellem s whole blood and Salzman s methods were compared a significant correlation between the two was found ($P < 0.01$) The relationship is illustrated in Fig 1

The relationship between Wright s and Salzman s method is illustrated in Fig 2 The correlation was significant ($P < 0.05$)

On the other hand when comparing Wright s and Hellem s whole blood methods (Fig 3) the

Table II *Methodological results from healthy subjects for each of the methods employed in the study*

Method	Wright	Salzman	Hellem	Hellem PRP/ADP
No of subjects	50	50	50	50
Mean adhesive value	29	44	25	43
S.E. ±	1 43	2 44	1 56	2 5
S D	10	17	11	17
Methodological error (n=20)	6	4	4	5

correlation was found to fall short of statistical significance (0 05 < P < 0 10)

Plasma versus whole blood methods

No significant correlation could be found when Hellem s PRP/ADP method was compared with Wright s Salzman s and Hellem s whole blood methods These relationships are shown in Figs 4–6

DISCUSSION

Four methods of estimating platelet adhesiveness have been compared Methodological details have been presented Minor differences exist between these and the original descriptions Citrate replaced heparin in Wright s rotator test In Salzman s method a modification was introduced as in our experience a longer glass bead column with beads of a total weight of 2 5 g rather than 1 0 g together with a shorter but well defined collection time of 35 sec reduces the methodological error

Our mean adhesion values for Hellem s whole blood method (Table II) are lower than those obtained by Hellem (9) and Ödegaard et al (25) This difference may at least in part, be due to the slightly shorter contact time used and the differences in the type of beads employed Contact time has been shown by Cronberg (5) to be one of the most important variables in platelet adhesion estimations

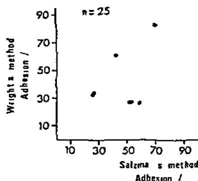


Fig 2 Relationship between Wright s rotating bulb and Salzman s methods.

In the present study when comparing whole blood methods good correlation was found between Salzman s and Hellem s methods Both these methods involve the passage of whole blood through glass bead filters but differ in delay and contact times temperature and in the fact that no anticoagulant is required for Salzman s method (Table I) A significant correlation at the 5% level was also obtained between Salzman s and Wright s methods On the other hand the relationship between Hellem s and Wright s whole blood methods fell short of statistical significance Our findings are therefore in agreement with those of O Brien and Heywood (19) who used their own

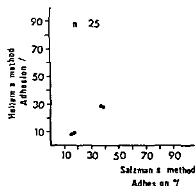


Fig 1 Relationship between Hellem s whole blood and Salzman s methods

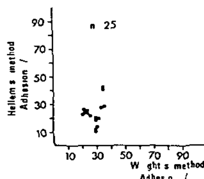


Fig 3 Relationship between Hellem s whole-blood and Wright s rotating bulb methods

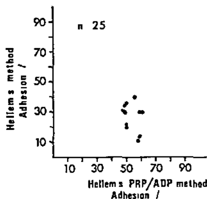


Fig 4 Relationship between Hellem s whole blood and platelet rich plasma/adenosine diphosphate methods

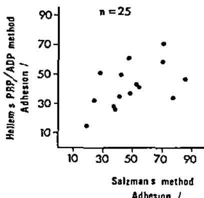


Fig 6 Relationship between Hellem s platelet rich plasma/adenosine diphosphate and Salzman s methods

modification of Hellem s whole blood method but differ from the highly significant degree of cor relation found by Hirsh et al (11) in their com parison of these two methods The lack of cor relation between these two whole blood methods for platelet adhesiveness would thus adequately explain the diverging results in diabetics reported by Bridges et al (3) who using Wright s method found raised adhesiveness whereas Ödegaard et al (25) using Hellem s whole blood method found low values

In the present study no correlation was found between any of the three whole blood methods investigated and Hellem s PRP/ADP method The lack of correlation between Hellem s whole blood and PRP/ADP methods is of particular in terest as these only differ in delay time (Table I) and in that erythrocytes provide adhesive sub stance (ADP) in the former while a well defined quantity of ADP is added for adhesion in the latter It should be pointed out that like Hellem (9) we found no adhesion when PRP only was passed through the glass bead column

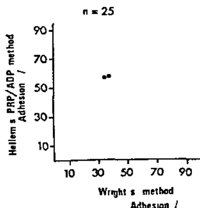


Fig 5 Relationship between Hellem s platelet rich plasma/adenosine diphosphate and Wright s rotating bulb methods

The lack of agreement between Hellem s PRP/ ADP method and all whole blood methods in vestigated may well explain some divergent re sults previously reported Thus the findings of increased platelet adhesiveness found by McDonald and Edgill (14) using Wright s method in subjects with ischaemic heart disease may not be in contrast with Rozenberg s and Stormorken s findings (20) who using Hellem s PRP/ADP method found no evidence of increased platelet adhesiveness in a similar group of subjects

The lack of significant correlations between the whole blood and the plasma methods also points to the role of the red blood cells in whole blood systems for platelet adhesion That erythro cytes play an essential part in platelet adhesion was demonstrated by Hellem (9) who found a sub stance R in red blood cells which at very low concentrations induced platelet adhesion Factor R was subsequently identified as ADP by Gard ner et al (7) The role of the erythrocytes re ceived further emphasis when Ödegaard et al (25) increased whole blood adhesion in patients with diabetes by replacing their red cells by those from healthy controls who had higher whole blood adhesive values Caspary (4) showed that small numbers of erythrocytes could give high adhesive values using Wright s method and Harrison and Mitchell (8) found that if ADP was removed by enzymatic phosporylation from a whole blood system adhesiveness would fall to that of PRP alone in Wright s method Further quantitative

studies to elucidate the relative role of the erythrocytes in platelet adhesion in whole blood systems are thus indicated

ACKNOWLEDGEMENTS

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BLADDER TUMOURS INDUCED BY CHLORNAPHAZINE

A Five year Follow up Study of Chlornaphazine treated Patients with Polycythaemia

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Abstract Among 61 polycythaemic patients treated with chlornaphazine ten developed bladder tumours Atypical cells have been demonstrated in the urines of another five The induction period for tumour development was from approximately 3 to 10 years rather independently of the total dose Therefore a careful follow-up over a period of many years is necessary for patients who have received this treatment, even for low total doses The most important link in the follow up is urinary examination for tumour cells

The carcinogenic effect of betanaphthylamine is well known Its derivative dichloro-diethyl beta naphthylamine (chlornaphazine) was in clinical use from 1948 to 1963 Owing to the inhibitory effect of this substance on haemopoiesis it was utilized to some extent in the management of polycythaemia

During the period from 1954 to 1962 a total of 61 patients with polycythaemia were treated with chlornaphazine in the Medical Isotope Department of the Finsen Institute Copenhagen But after studying the causes of death among Danish polycythaemic patients we reported in 1962 two cases of bladder carcinoma and one of renal carcinoma among 26 patients who had been treated with high doses of chlornaphazine and emphasized the likelihood of its carcinogenic effect (4) As a result this treatment was discontinued in a large number of our patients in 1961 and in 1962 we definitively stopped using the drug

Since 1963 chlornaphazine has been prohibited in Denmark In 1964 seven cases of bladder tumours were reported among the above mentioned patients from the Finsen Institute (7) At the same time Videbæk (9) described two cases among patients with Hodgkin s disease also from the Finsen Institute

The following is a report on the conditions on 1 March 1968 more than five years after chlornaphazine was definitively withdrawn During this period another three cases of bladder tumours have occurred and abnormal cells have been demonstrated in the urines of a further five patients

MATERIAL

The material comprised 61 patients 34 males and 27 females On 1 March 1968 27 were still alive The first case of bladder tumour was demonstrated as an accidental autopsy finding in a patient who died in another hospital in 1958

Table I gives some data on the 10 patients in whom bladder tumours developed under the influence of chlornaphazine

It must be emphasized that the chlornaphazine doses given in Table I are approximate Anorexia, nausea vomiting and epigastric pressure were common side effects and it is apparent from many of the case record that the patients reduced the dose of their own accord or made periodical pauses in the treatment.

The first sign of bladder tumour was usually observed during treatment In many cases it was haematuria. However microscopic haematuria is not uncommon in patients with polycythaemia partly because of generalized hyperaemia and partly due to a tendency to lithiasis Moreover a number of the patients were on anticoagulant therapy Thrombocytopenia was also present in one case Furthermore it must be stressed that the patients were of an age when cystitic complaints are on the whole common so that it is difficult to state the exact time of the first symptom Consequently we define the induction period as the time from the first day of treatment until bladder tumour was diagnosed

Table III *Twenty seven patients who have died without signs of bladder tumour*

Pat no	Sex	Total of chlornaphazine (g)	Duration of treatment (y)	Duration of pauses in treatment (mo)	Other treatment	Died mo after discont of chln therapy	Cause of death	Age at death	Autopsy
1	o	180	1958 61	7	^{32}P 8 6 mC	55	Blast leukaemia	68	+
2	♀	175	1958 61	0	^{32}P 8 5 mC	23	Blast leukaemia	47	+
3	♂	145	1958 61	20	^{32}P 10 0 mC	2	Blast leukaemia	72	+
4	♂	105	1959-62	18	^{32}P 13 0 mC	18	Blast leukaemia	65	+
5		118	1957 61	20	^{32}P 10 5 mC	5	Blast leukaemia	53	–
6	♂	130	1958 59	0	^{32}P 22 5 mC	0	Anaemia gravis	69	–
7	♂	100	1960-6?	0	0	?1	Hypernephroma	55	–
8	♀	100	1959 61	11	^{32}P 14 0 mC	0	Mesenteric thromb	73	–
9	♂	1 0	1954 57	36	^{32}P 12 0 mC	0	Pleuropneumonia	64	–
10	♂	80	1959-60	0	^{32}P 8 0 mC	61	Myelofibrosis	57	+
11	♂	80	1961-62	0	^{32}P 19 0 mC	33	Suicide	63	–
12	[illegible]	55	1957 58	1	^{32}P 8 0 mC	21	Leukaemoid react. (Late stage of poly cythaemia)	54	+
13	o	46	1957-61	40	^{32}P 11 0 mC	7	Miliary tuberc.	68	+
14	o	53	1954 56	1[illegible]	^{32}P 13 0 mC	[illegible]	Myelofibrosis	43	+
15		53	1959 61	24	^{32}P 4 0 mC Busulfane	66	Cystopyelitis	89	+
16	o	48	1960-61	0	^{32}P 3 0 mC Busulfane	50	Leukaemoid react (Toxic react)	59	–
17	♂	35	1955	0	^{32}P 10 0 mC	44	Pulm. haemorrh	55	+
18	♂	34	1958-61	34	^{32}P 13 0 mC Busulfane	57	Arterioscl heart dis.	76	–
19	♂	8	1958	0	0	0	Coron thromb	59	–
20	o	[illegible]5	1957 58	7	^{32}P 19 0 mC	0	Coron thromb	7	–
?1		25	1959	0	^{32}P [illegible]0 mC	4	Leukaemoid react. (Late stage of poly cythaemia)	64	+
22	o	19	1959	0	^{32}P 10 0 mC	38	Arterioscl. heart dis.	75	–
[illegible]3	♂	15	1960	0	^{32}P 7 0 mC	36	Decompens. heart dis.	58	–
[illegible]4	o	12	1957 58	0	^{32}P 1? 0 mC Busulfane	76	Pancytopenia	75	+
25	[illegible]	10	1957	0	^{32}P 9 0 mC	41	Arteriosci	73	+
26		8	1954	0	^{32}P 8 9 mC	[illegible]	Myelofibrosis	69	+
?7	♂	7	1955 57	19	Busulfane	74	Thrombocytopenia	61	–

therapy may have been operative is uncertain There are no common features regarding occupation Some indications of smoking habits are given in Table I Some patients were heavy smokers one did not smoke at all and cigarette-smoking was not predominant Lastly it must be emphasized that polycythaemia does not predispose to the development of bladder cancer (4 8) ^{32}P therapy has hardly played any role in the development of the bladder tumours (4)

Table III lists the data on the deceased patients in whom no signs of bladder tumour were observed An autopsy report is available for 15 subjects Another seven patients died in hospitals In these cases there had been no objective signs of bladder tumour Five patients died in their homes One died in 1958 after 5 months of chlornaphazine therapy Another patient died shortly after operation for hypertrophy of the prostate It is apparent from the operative report that the bladder mucosa showed no changes suggesting tumour As far as the remaining three patients are concerned urinary examination showed no abnormal cells

Thus it is not likely that bladder cancer has occurred in further patients

It is evident that some patients received very high doses of chlornaphazine without bladder tumour developing That the patients did take the drug is apparent from the effect on the haemoglobin white blood counts and platelet counts The same phenomenon is known from animal ex

periments in which tumours do not develop in all animals of a series in spite of intensive exposure to a carcinogen (1)

Boyland (2) has described the developmental mechanism of bladder tumour following administration of betanaphthylamine and its derivatives The actual carcinogens are orthoaminophenols which form in the liver but are immediately detoxicated by binding to glucuronides sulphates and phosphate esters and are transported in the organism in a detoxicated form In the urinary tract they split under the influence of betaglucuronidase and this releases the carcinogen It might be supposed that the concentration of betaglucuronidase could play a role According to Fripp (6) it is derived partly from the bladder epithelium and partly from renal tubular activity

A complete survey on the role of carcinogens in *the development of bladder cancer is given in* J Clemmesen Statistical studies in malignant neoplasms (5)

From Tables I and III it is apparent that in seven of the present 61 patients blast leukaemia developed All these patients had been treated with high total doses of ^{3} P As previously reported (4) blast leukaemia is even more common among patients who have been treated with ^{3} P as well as with chlornaphazine Thus it is probable that chlornaphazine has played a role in the development of leukaemia

Incidentally it was because of the risk of leukaemia after ^{3} P therapy that chlornaphazine was introduced in the treatment of polycythaemia Treatment with venesection alone had often given disappointing results and without treatment the prognosis was poor

Owing to the long induction period it must be assumed that further cases of bladder tumours will occur among chlornaphazine treated patients and that all such patients should be frequently examined even if they have received only a low total dose

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Acta med. scand Vol. 185 pp 139-143 1969

PERMANENT CARDIAC PACING

A follow up study of 88 patients

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Abstract A follow-up study of 88 patients on perma nent cardiac pacing is reported. The observation time was from one month to six years, on average 1 / years. A-V block was seen in 85 S-A block in three patients. The etiology was primary A-V block in 57 coronary heart disease in 19 and other heart diseases in 12. Adams-Stokes attack was observed in 73 patients The mean age was 69 years. Twelve patients were primarily treated with epicardial and 76 with endocardial elec trodes. In five patients a sh ft was made from epicardial to endocardial electrodes The mortality in the first year was 13% the total mortality 22% In patients with primary A-V block the total mortality durin, the ob- servation period was 9% in coronary heart disease 47% In the authors opinion endocardial pacing is a reliable method and the one which should be preferred. A regular ambulant control is very important, preferably in a specialized pacemaker-clinic

Permanent cardiac pacing is now a well established therapy for patients with permanent or recurrent atrioventricular block (2 4) The main indications are Adams-Stokes attacks or extreme bradycardia. In the practical performance of cardiac pacing it is possible to choose between different methods i e epicardial or endocardial electrodes fixed rate on demand or atrial trig gered pacemakers and to choose between a mul titude of different designs of electrodes and pulse generators from various manufacturers Many practical details in routine long tim- cardiac pac ing have yet to be settled

We here report our experiences in permanent cardiac pacing in 88 patients treated from 1961 to 1967 in order to elucidate some prognostic ques tions and practical details concerning this therapy Endocardial (transvenous) pacing has been used as the only method from June 1964

MATERIAL

In the period 1961-1967 we have treated 88 patients with permanent cardiac pacing. The mean age of the pa tients was 69 years. The number of patients in the dif ferent age groups appears from Table I The observation time for each patient appears from Fig. 1 The average observation time until January 1 1968 or until death was one and a half years.

Primary atrioventricular block was seen in 57 pa tients. Coronary heart disease with typical angina pectoris or previous myocardial infarction was found in 19 pa tients. In the remainder the etiology was rheumatic heart disease in seven, luetic heart disease in two colla enos s in two and a syndrome of paroxysmal atrial flutter and ventricular asystole in one patient.

Indications

The indication for cardiac pacing was Adams-Stokes at tacks in 73 cases, of whom 72 had atrio-ventricular block and one sino-auricular block. In 14 patients 13 with A-V block and one with S-A block, the indication was severe bradycardia with cardiac failure or cerebral symp- toms. One patient with paroxysmal atrial flutter followed by post-paroxysmal S-A block, was successfully treated with a combination of a permanent pacemaker and pro- pranolol.

METHODS

The first twelve patients, all treated before June 1964 had Elema epicardial electrodes implanted by thoraco- tomy One d fferent, one indifferent and one spare elec trode were implanted. Eight of these 1 patients are alive three of them still have epicardial electrodes, while in five a shift has been made to endocardial electrodes. The indication for this shift to endocard al electrodes was electrode failures in three cases and infection in two

In 76 patients exclusively intracardial electrodes have been used. Including the fi e patients who had epicardial electrodes replaced by endocardial the total number of patients with endocard al pacing is 81 In 15 emergency

Table I *Number of patients with permanent cardiac pacing in different age groups*

Age (y)	< 50	50–59	60–69	70–79	80–	Total
Men	2	9	13	17	3	44
Women	1	5	10	21	7	44
Total	3	14	23	38	10	88

cases the positioning of the electrode was guided by ECG monitoring (1) in the other cases TV monitoring was used. In 67 cases the electrode was introduced through the external and in 14 cases through the internal jugular vein. The external end of the electrode was passed behind the clavicle and led subcutaneously to the lower iliac region, distal to the proposed site of battery implantation. A long hollow needle designed for this purpose was used. An external pacemaker was used for 1–2 weeks to secure an appropriate threshold stimulation value before implantation. All the patients are followed up regularly at three-month intervals.

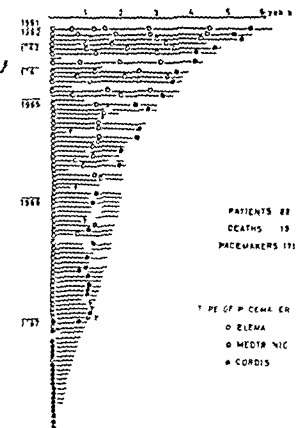


Fig. 1 Patients on permanent pacing. General survey indicating observation time, deaths, types, numbers and function time of pacemakers.

Table II *Prognosis in relation to etiology of heart disease in patients on permanent cardiac pacing*

Etiology	No of pats	Deaths	Cause of death: Related to the pacemaker treatment	Cause of death: Other causes
Primary A–V block	57	5	2	3
Coronary heart disease	19	9	1	8
Rheumatic heart disease	7	2	1	1
Luetic heart disease	2	1	0	1
Collagenosis	2	2	1	1
Paroxysmal flutter	1	0	0	0
Total	88	19	5	14

RESULTS

Mortality

The mortality in the first year was 13% (8 out of 63 patients) and the total mortality during the observation period was 22%. Of 27 patients under observation for more than two years two have died. Causes of death and mortality related to the etiology of the heart disease appear from Table II. Deaths directly related to pacemaker therapy include three fatalities from "run-away pacemaker" and two operative casualties. The other fatalities were sudden death (2), heart failure (2), myocardial infarction (3) and non-cardiac diseases (7). In patients with coronary heart disease the mortality during the observation period was 47%, in patients with primary A–V block only 9%. It thus appears that the prognosis depends on the primary etiology of the heart disease.

Electrode failures

In the first two weeks after introduction of the electrode pacemaker failure due to dislocation or poor positioning of the endocardial electrode was seen 12 times, all corrected by repositioning of the electrode.

Spontaneous late dislocation of the endocardial electrode after implantation of the pulse generator has been observed in four cases, after 1 1/2, 2, 4 and 7 months respectively. In three patients who were discharged with an external pulse generator the electrodes were accidentally pulled out. In the last years we have implanted all the pulse generators after 1–2 weeks to avoid this compli-

Table III *Number and type of pulse generators Function time and indications for change*

Type	Total no implanted	Indication for change of pulse generator (in brackets average function time)				Still functioning	Patient dead
		Routine	Failure of pulse generator	Fistula or infection	Electrode failure		
Elema 137	19	1 (15 mo)	15 (12 mo)	1 (6 mo)	2 (6 mo)	0	0
Elema 139	89	50 (14 mo)	17 (14 mo)	1 (10 mo)	3 (9 mo)	8 (11 mo)	10 (1[illegible] mo)
Ventricor	49	0	1 (1 mo)	1 (6 mo)	1 (9 mo)	45 (4 mo)	1 (5 mo)
Ectocor	3	0	0	0	0	3 (1 mo)	0
Medtronic 5870-C	12	0	0	1 (1 mo)	0	10 (4 mo)	1 (1 mo)

cation In three cases the electrode was accidentally pulled out during operation for change of pulse generator

In no case has a wire break been observed In three cases damage to the insulation of the electrode has resulted in pacing failure

Failing contact between pulse generator and electrode has resulted in five operations in three of them (2 Elema 1 Cordis) complete pacemaker failure occurred because the electrode had loosened from the fastening screw in the pulse generator

Instrumental failure

Types and numbers of pulse generators used their function time and indications for replacement appear from Table III

Until August 1964 we used *Elema 137* pacemakers In these the pulse rate gradually decreased when the batteries were exhausted Signs of battery failure appeared after an average of 12 months For the next period until January 1967 we used Elema 139 pacemakers which were electively changed after 15 months but after some episodes of *run away pacemaker"* we reduced the interval for routine change for these pulse generators to 12 months

We have seen eight episodes of "run away pacemakers all of them being of type Elema 139 Three of the patients died The episodes appeared after 8–21 months (Table IV)

Exit block

Pacing failure related to an abnormally high myocardial stimulation threshold i e exit block was observed once the pacing threshold found at re-operation was 10 V In two cases both with Ventricor pacemakers pacing failure was probably due to a combination of a short circuit near the pacemaker and a rising threshold value Inadequate sealing of the connection to the negative electrode resulted in a short circuit which drew current from the pacemaker In both the failure occurred one month after implantation Threshold measurements at re-operation showed values of 2 and 4 volts respectively

Other complications

In four instances pulse generators had to be removed due to fistula or wound necrosis Three of the patients were old and in a poor general condition the fourth had an infected hematoma with fistula

Competition between pacemaker rhythm and the patient s own heart rhythm has been noted in 43 cases (50 %) In only a few cases did this lead to subjective discomfort

Thromboembolism was recorded in only one patient, in whom a small pulmonary embolus appeared No instance of sepsis was seen. Per

Table IV *Run away pacemakers*

Time after implantation (mo)	Rate	Outcome
8	150	Survived
9	116	Died
11	1 0	Survived
14	160	Survived
14	184	Survived
14	2 0	Died
15½	2 0	Died
21	400	Survived

Congress Announcements

The International Symposium on Pulmonary Circulation will be held in Prague June 10 to 13 1969

Secretariat Czechoslovak Medical Society J. E. Purkyně International Symposium on Pulmonary Circulation Sokolská 31 Praha 2 Czechoslovakia

The Sixth Conference of the European Dialysis and Transplant Association (EDTA) will be held in Stockholm Sweden June 27 to 28 1969 in connection with the International Congress of Nephrology

Information from Dr R Natusch Gesellschaft für Nephrologie der DDR Schumannstrasse 20 21 104 Berlin

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MONOAMINE OXIDASE ACTIVITY IN THE HUMAN SMALL INTESTINE

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Abstract Monoamine oxidase (MAO) activity in the epithelium of the normal human small intestine was found to be very high 780 nmol IAA/mg h which is higher than the MAO activity in other tissues according to earlier studies After nialamide treatment (150 mg/day orally) for two weeks the activity was reduced by 85 This is about the same as that seen after pargyline and isocarboxazide treatment and cannot be the explanation of the lesser complications with nialamide Merely the decrease of MAO activity in the epithelium of the small intestine is probably a very important factor in the development of the circulatory reactions from which patients treated with monoamine oxidase inhibitors suffer after ingesting certain food containing monoamines

The measurement of MAO activity in the intestinal mucosa was found to be a useful method for clinical work

Several circulatory reactions may result from the ingestion of cheese (2) broad beans (7) wine (6) and yeast extracts (3) by patients treated with monoamine oxidase (MAO) inhibitors Some of these reactions are fatal Various biogenic amines are thought to be the causative agents of these reactions which have appeared only in association with some MAO inhibitors However patients treated with nialamide have not suffered from severe circulatory reactions Only some cases of headache are described (5)

Parenteral tyramine in unanaesthetised and dopamine in anaesthetised rabbits did not cause circulatory reactions however (1) Thus it is possible that also the route of administration may be an important factor augmenting the effect of amines by MAO inhibition Levine and Sjoerdsma (9) have demonstrated that pargyline and isocarboxazide cause a clear decrease in MAO activity in the epithelium of the human small intestine Clinical reactions frequently appear during treatment with these MAO inhibitors

The purpose of the present study was to investigate whether the lesser effect of nialamide on MAO activity in the epithelium of the small intestine would possibly clarify the rare clinical reactions of nialamide and to examine the usefulness of determination of the intestinal MAO activity in the clinical material

MATERIAL AND METHODS

The normal series consisted of ten patients who had no verifiable disease of the small intestine or liver This group included four males and six females Their mean age was 50 3 years The group treated with nialamide (Niamid®) comprised eight patients three males and five females, and their mean age was 46 1 years The dose of nialamide was 50 mg administered orally three times daily for two weeks Jejunum biopsies were obtained by the suction method (4) and every specimen was immediately deep frozen to −20 C The specimens were weighed just before MAO determination by the method of Lovenberg et al (10) The specimens weighed ~18 mg Their incubation time was 60 min at +37 C MAO activity was calculated in nanomoles of IAA formed from tryptamine incubated with 1 mg of tissue for one hour In the normal material 10^{-} *M* phenprazine chloride inhibited over 90 and 10^{-} *M* semicarbazide chloride only 8 of the activity

RESULTS

The MAO activity in the epithelium of the human small intestine in the normal and the nialamide treated groups is shown in Table I The difference between the normal material and the nialamide treated group was highly significant (p 0 001)

Table I *MAO activity of the intestinal epithelium of normal human controls and nialamide pretreated patients*

Group	Controls			Nialamide pretreated		
	All	Women	Men	All	Women	Men
MAO activity						
(nmol IAA/mg h)	25 0	8 5	27 2	4 3	4 4	4 1
S.E	3 .	5 1	3 0	1 0	1 5	1 0
n	10	6	4	8	5	3
Inhibition percentage				85	85	85

DISCUSSION

The measurement of MAO activity repeatedly in vivo is possible only in the epithelium of the small intestine or platelets (11) We consider that not only the MAO inhibition effect alone but also the route of administration is of importance in the circulatory reactions We therefore measured the MAO activity in the epithelium of the small intestine At the same time we conclude that the measurement of MAO activity from the intestinal mucosa is a method suitable for clinical use and that the intestine may be the best organ for samples that reflect the MAO activity in the whole organism

We discovered like Levine and Sjoerdsma (9) a very high MAO activity in the epithelium of the normal human small intestine In all other tissues this activity was found by measurement to be smaller in the liver for instance it was 25 nmol IAA/mg/h (18) This indicates that the MAO of the small intestine may play an important role in the detoxification of food and drugs

The percentage of inhibition by nialamide was 85% which is very significant considering the small doses of nialamide used in our experiments The results agree well with those of Levine and Sjoerdsma (9) which they obtained with pargyline (100 mg/24 h) and isocarboxazide (30–40 mg/24 h) 87–92% and 78–87% respectively This indicates that the effect of nialamide as MAO inhibitor in the epithelium of the small intestine is about the same as that of pargyline and isocarboxazide After nialamide treatment the penetration of biogenic amines into the general circulation is not less than after pargyline and isocarboxazide treatment This does not explain the reason for the lower circulatory reactions when nialamide is used

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METASTATIC CALCIFICATION OF SOFT TISSUE ON OVERDOSAGE OF VITAMIN D

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Abstract Nephrocalcinosis and renal insufficiency in a case of vitamin D intoxication are described in order to emphasize the risk of overdosage in careless or uncontrolled administration of vitamin D and to report a successful form of treatment in which sodium phytate may have played an important part The case indicates that it may be possible to reduce the serum calcium level by oral administration of sodium phytate and that—with the patient at home—this level can be kept low for a long time by means of a low calcium diet It is also indicated that a distinct roentgenologic regression of calcification of the renal tissue with simultaneous improvement of renal function is quite possible

The antirachitic effect of irradiated ergosterol was demonstrated in the mid 1920s by Hess et al (9) and by Steenbock and Black (24) The toxic effect of this substance when given in a large amount was shortly afterwards shown on laboratory animals and at the end of that decade Putschar (16) reported the first case of death from vitamin D overdosage

During the 1930s high doses of vitamin D became increasingly common as therapy in a number of diseases predominantly those involving the skin and the joints and a large number of cases with intoxication symptoms were reported By the end of the 1940s many deaths due to vitamin D intoxication had occurred After this period however it seemed—from the rather few cases reported with more serious complications—that greater care was being taken in the prescription of vitamin D in large doses To judge from our experience in this clinic vitamin D intoxication appears to have again become more common during recent years In the following the most noteworthy of our cases with nephrocalcinosis and renal insufficiency will be described partly to emphasize the risk of overdosage in careless or uncontrolled administration of vitamin D and partly to report a successful course in which treatment with sodium phytate may have played an important part

CASE REPORT

Female born 19[illegible] VIII para Goitre since the beginning of the 1940s No signs of thyrotoxicosis In 1959 a bilateral resection was performed Postoperatively falling blood calcium values at the lowest 2.9 mEq/l symptoms of tetany began Treatment with Fortedol (vitamin D) in a dose of [illegible]0 drops × 3 (= [illegible]70 000 IU) per day for 10 days was given after which the serum calcium was 6.6 mEq/l. The Fortedol treatment was then discontinued and after two more weeks the patient was discharged in good general condition and with a serum calcium value of 5.2 mEq/l After two weeks at home without therapy she was readmitted to the hospital for a check up The calcium level was then found to have fallen to 4.1 mEq/l No clinical sign of threatening tetanus She was discharged with a prescription for Fortedol 5 drops × 2 (= 45 000 IU) per day and told to return for a further check up after one month However she failed to come She obtained new prescriptions for Fortedol from a local doctor and continued with the dose of 45 000 IU daily

In October 1965 she attended the outpatient department of the medical clinic having been referred for analysis of anaemia The patient had been taking the above mentioned Fortedol dose daily since July 1959 Since July 1965 she had suffered from increasing tiredness breathlessness headache nausea, vomiting and diarrhoea. During the last 3–4 months her weight had decreased by 7–8 kg Admitted as inpatient for investigation BP 160/85 Hb 7.3 g serum creatinine 3.4 mg% Serum calcium at repeated tests 8.5–9.6 mEq/l other electrolytes within normal limits, specific gravity in concentration test 1.010

ECG showed sinus rhythm and ST T changes corresponding to the left ventricle X ray of urinary tract revealed a large number of grain-sized calcifications in the medullar regions of both kidneys which were of normal size and shape (Fig 1 *a*)



Fig 1 X ray of urinary tract before (*a*) and after (*b*) treatment with low-cal ium d et and sodium phytate *a* shows a large number of grain sized calcifications in medullar re ons of both kidneys. In *b* there is recession of the cal ifications in both kidneys.

The patient was treated with a low-cal ium diet and sodium phytate 1 5 g 6 After ten days of treatment the serum cal ium was normal The patient was discharged with instructions for low-cal ium diet During the next 3 weeks the serum calcium was 5 7–5 8 mEq l and sodium phytate 1 5 g 6 was prescribed for a further 3 weeks After this the serum calcium was normal and has since remained below 5 0 mEq l Low calcium diet wa continued

In *July* 1966 certain signs of threatening tetany were observed Readmitted Serum calcium was 3 9 mEq l serum creatinine 1 8 mg* BI 149 90 Concentration te t showed a maximal specific gra ity of 1 013

In September 1966 in add tion to paresthesia and prickling in her arms and legs and cramps in the muscles of the hand and forearms a positive Trousseau was also noted Serum calcium was 3 8 mEq l erum creatinine 1 6 mg Returned to normal diet The tetany symptoms then disappeared rapidly In December 1966 the serum calcium was 3 9 mEq l serum creatinine 1 6 mg and Hb 1_ g X ray of urinary tra t showed regres ion of the smaller calcifi ations in both kidneys (Fig 1 *b*)

The patient is now in excellent condition Attention has been drawn to this case by Ask Upmark (1)

DISCUSSION

Most reports from the 1930 s and the 1940 s on vitamin D intoxication dealt with children and several uthors supposed that children were more easily disposed to calcification of the soft tissues on overdosage of this substance than adults How ever ny difference in this respect may be due

Table I *Earlier reported metastatic calcification in soft tissue on overdosage of vitamin D*

	Age	Sex	Vitamin D dosage	Duration of therapy	Localization of soft tissue
Danowski T S Winkler A W and Peters J P (1945)	57 y	♀	150 000–200 000	6 y	
			450 000 IU	1 mo	Calcification in soft tissue around several joints
Bauer J M and Freyberg R H (1946)	3 y	♀	100 000–500 000 IU	At least 1 y	Calcification in the kidneys heart lungs subcutaneous tissues and arterial vascular system
Freeman S Rhoads P S and Yeager L B (1946)	49 y	♀	100 000–300 000 IU	About 2 y	Calcification in subcutaneous tissue around several joints
Mulligan R M (1946)	44 y	♂		6 mo	Calcification of heart lungs gastric mucosa kidneys, pancreas and numerous blood vessels
Bevans M and Taylor H K (1947)	63 y	♀	180 000–200 000 IU	2 ¼ y	Calcification of periarticular and subcutaneous tissues lungs heart and kidneys
Hyde L and Hyde B (1947)	59 y	♂	200 000 IU	17 mo	Calcification of lung subcutaneous calcification around several joints
Kaufman P Beck R D and Wiseman R D (1947)	63 y	♂	150 000–200 000 IU	14 mo	Calcification around several joints and of renal and iliac arteries
McLean G and Lebo L (1948)	56 y	♂	150 000–180 000 IU	3 y	Calcification around several joints and of arteriovascular system
Smith W H (1963)	21 y	♀	100 000–200 000 IU	3 y	Calcification in the main bronchi medium sized arteries and kidneys

to the fact that the doses of D vitamin given are generally larger for children in relation to body weight Metastatic calcification of soft tissue which is caused most probably by vitamin D overdosage has been described by several authors The present paper and the tabulated survey of such cases given below concern only adults who had no complicating diseases that could be suspected of producing a special hypersensitivity to vitamin D and no disturbed calcium metabolism as part of their clinical picture —Thus in connection with vitamin D therapy Livingstone and Walker (13) described widespread metastatic calcification of the soft tissues in a woman with sclerodermis and Wells and Holley (25) in a patient with Paget s disease For reports on calcinosis as a complication of vitamin D administration in children reference may be made to Bauer et al (2) Ross (19) and De Wind (5) It is also considered possible for D hypervitaminosis to occur in children despite the absence of exogenous intoxication In cases with what is known as idiopathic hypercalcaemia neither nephrocalcinosis nor other kinds of soft tissue calcification are unusual

As shown in Table I in the mid and late 1940 s metastatic calcification of soft tissues was reported in a small number of adult patients as a complication of the high dosage of vitamin D Since then we have only found descriptions of a few isolated cases Probably this is partly due to the fact that treatment of rheumatoid arthritis in particular with vitamin D has now been discontinued or only occurs to a very limited extent but also because the numerous earlier reports of serious complications has caused a certain restriction with regard to dosage and duration of treatment and special care is taken to observe any possible symptoms of intoxication

In earlier reports calcification of soft tissues on overdosage of vitamin D has been described mainly in the kidneys bronchi arteries and periarticular tissue Renal calcification has occurred predominantly in arteries and more rarely in tubules and glomeruli (10) Signs of prolonged renal insufficiency have been observed in vitamin D intoxication without complicating calcification of renal metastatic soft tissue The most common symptoms of such intoxication are tiredness sickness vomiting diarrhoea polyuria loss of weight muscular weakness and headache Our patient showed all these symptoms on her first visit to our clinic after having taken about 45 000 IU vitamin D daily for six years a dose that is low compared with those of patients previously de

scribed with metastatic calcification of soft tissues *More distinct symptoms had been noted* however during the last 3–4 months before treatment Slight gastro-intestinal disturbances are common however in overdosage of vitamin D and it is obviously of importance to pay attention to such symptoms If the vitamin D dose is reduced rapidly or discontinued altogether it is possible to avoid the serious complication of metastatic calcification of soft tissues (which if the kidneys are involved can be fatal) Our patient exhibited isosthenuria and normal sediment As early as 1948 Howard and Meyer (10) pointed out that low specific gravity of the urine and loss of formed elements in the sediment are characteristic of this disease

Corticosteroid therapy is still the current form of treatment in hypercalcaemia due to sarcoidosis overdosage of vitamin D and infantile hypercalcaemia Our patient was treated with the sodium salt of phytic acid which when taken orally increases calcium excretion in the faeces this has been attributed to reduced intestinal absorption of calcium probably due to the formation of unabsorbable calcium phytate At the same time the calcium excretion in the urine decreases Henneman et al (8) in 1956 were the first to call attention to the capacity of sodium phytate to normalize the serum calcium in patients with hypercalcaemia due to sarcoidosis Roelsen and Paulsen (18) demonstrated excellently how the calcium excretion in the urine decreased during treatment with sodium phytate both in a patient with hyperparathyroidism and in one who had been given as prophylaxis against parathyroprival tetany large doses of vitamin D and also—though on a low level—in a patient whose hypercalcaemia had occurred in association with generalized sarcoidosis The serum calcium became normal in the two latter patients whereas in the first patient mentioned it was not influenced to any noteworthy extent over a long period Owing to technical difficulties Roelsen and Paulsen did not determine the faecal calcium but Henneman et al (6) had demonstrated previously that calcium excretion in faeces increases in patients with sarcoidosis and hypercalcaemia when sodium phytate is given orally

In our patient the sodium phytate probably contributed greatly to the reduction of the serum calcium level which began only a few days after treatment with this substance was started and to *the normalization that* was noted after about one week However no such rapid improvement was seen as regards the numerous grain sized calcifications in the medullar regions of both kidneys or as regards renal function Regression of these calcifications and improvement in renal function were gradually observed however during the follow up examinations To what extent the low-calcium diet contributed in effecting these improvements is difficult to decide Earlier authors have reported spontaneous regression after discontinuation of vitamin D treatment Shelling and Asher (21) and Selye (20) indicate that the metastatic calcifications in the tissues are apt to develop at sites of local injury

Opinions differ on the toxicity threshold of vitamin D Bauer and Freyberg (2) claimed that this threshold was generally considered to be at 20 000 IU per kg body weight and day but Reed et al (12) reported toxic manifestations in an adult at 1000 IU per kg body weight and day It seems certain however that there are great individual variations in this respect dependent on several contributory factors Not only is the dose per kg body weight and day of importance but also *duration of treatment composition of diet* especially regarding its content of minerals and salts function of gastro-intestinal tract and endocrine system as well as the patient s age and vehicle of vitamin administration

The daily dose of vitamin D seems to be lower in our patient than in any other case with metastatic calcification of soft tissues due to overdosage with this vitamin which has been described on the other hand the duration of the treatment in our case was long We did not find any characteristic feature in the patient s diet No disturbances of the digestive tract seem to have occurred previously and the endocrine system apart from the thyroid (hyperthyrosis) and parathyroid (surgically removed) showed no signs of dysfunction An alcohol solution was used as the vehicle for vitamin D administration

In this connection—the genesis of metastatic calcification—Selye s theories on calciphylaxis are of great interest Selye has shown that tissues may be made sensitive to calcification and this sensitization he has named calciphylaxis During the period of sensitivity calcification may be induced by a number of challengers any of which

alone may be quite without effect According to McLean (14) Selye s calciphylaxis has four characteristics 1 the calcifer or the systemic sensitizing agent 2 the challenger of the vital mordant 3 the adjuvant or the topical activator of the vital mordant which potentiates the action of subthreshold amounts of the challenger and finally 4 the critical period which must elapse between treatment with the sensitizer and with the challenger Selye defines calciphylaxis as a condition of hypersensitivity in which—especially during the critical period after sensitization by a systemic calcifying factor (e g vitamin D or parathyroid hormone)—topical treatment with certain challengers (e g metallic salts) causes an acute local calcinosis followed by inflammation and sclerosis

Reduction of the renal function after overdosage of vitamin D has been reported several times Patients whose renal function was normal before vitamin D therapy have manifested after taking large doses over a long period albuminuria haematuria and increase of non protein nitrogen without apparent nephrocalcinosis It appears (23) as if the kidneys are exceptionally vulnerable to vitamin D and that the renal function has to be normal to permit long term therapy with a large dose of this vitamin Thus spontaneous regression of calcification of soft tissues due to overdosage of vitamin D does not seem to be altogether uncommon However we have found no previous description of a similar case in which the renal tissue calcifications regressed so beautifully As mentioned earlier it is difficult to estimate the importance of the calcium phytate in this respect but the reasons previously given favour the belief that it was of little or no importance Nor are we able to state with certainty the significance of the low-calcium diet in this connection It seems reasonable to assume however that it did have some influence since it was probably responsible for the rather low serum calcium level which persisted as long as the low-calcium diet was maintained It has certainly not been proved that a low serum calcium level is in itself of importance to regression of calcifications but it seems probable that such an effect can be ascribed to the maintenance of a constant low serum calcium over a long period

The case described here illustrates very clearly that long term therapy with high or rather high doses of vitamin D requires continuous supervision of the patient The blood calcium should be frequently checked and if signs of hypercalcaemia appear the vitamin D treatment should be discontinued or—as in prophylaxis against parathyroprival tetany—the dose should be reduced This case also indicates that it may be possible to reduce the serum calcium level by oral administration of sodium phytate and that—with the patient staying at home—this level can be kept low for a long time by means of a low calcium diet It is further indicated that a distinct roentgenological regression of calcification of the renal tissue with simultaneous improvement of renal function is quite possible

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HEREDITARY ABNORMAL MUSCLE METABOLISM WITH HYPERKINETIC CIRCULATION DURING EXERCISE

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Abstract Patients with a hereditary myopathy with paroxysmal myoglobinuria were studied in the chronic state of the disease They were characterized by muscle contractions of fairly normal strength and quite good endurance in exercise with small muscle groups, but a poor physical performance in exercis of som duration with large muscle groups

Several facts indicate that it was not muscular weakness but the circulatory capacity that was a main factor limiting the physical working capacity in exercise of some duration with large muscle groups The oxygen uptake was largely normal in relation to the work performed However e en light exercise on a bicycle ergometer caused tachycardia (approaching maximum heart rate) a high cardiac output (approaching, for the size of the individual maximum values in the normal range) and an abundant blood flow through the exercising legs Thus there were no signs of insufficient heart function but the utilization of the oxygen of the blood in the exercising limbs was low

The concentration of lactate and particularly of pyruvate in the blood and the lactate and pyruvate production increased more than in the controls for the slight work performed During exercise the lactate pyruvate ratio decreased and the calculated excess of lactate" was negative while it increased in normal subjects After exercise the excess lactate was higher in the patients than in the controls This indicates an abnormal muscle metabolism, probably a decreased capacity for pyruvate oxidation.

It is suggested that a metabolic disturbance caused an abnormally large production of metabolites in the working muscles resulting in muscular vessel dilatation by a local humoral effect. The muscles acted almost like a v shunts and as a consequence the patients had a hyperkinetic circulation i e a low O uptake in relation to cardiac output, a low a v O difference for mixed venous blood and for the venous blood of the exercising limbs.

A hereditary myopathy with paroxysmal myoglobinuria and abnormal glycolysis has been described by Larsson et al (23) The patients were characterized by a low exercise tolerance When performing light exercise with large muscle groups for a few minutes they had high pulse rate large ventilation and high cardiac output despite low oxygen uptake They also showed high concentrations of lactate and particularly of pyruvate in the blood The low tolerance for prolonged muscular exercise contrasted with the fairly normal muscle strength when performing muscle contractions of short duration

In this paper a more complete report than previously is given on data concerning exercise tolerance and some circulatory and metabolic changes observed in these patients

MATERIAL

The patients studied belonged to fi e families, A, B C D and E Nine of them, patients A 2 A 3 A 4 B 1 B 2 C 1 D D 3 and E 1 were examined more extensively Case reports of several of these patients and details about the clinical examinations were given in the previous paper (23) in which the case symbols were identical with those of the present paper None of the examinations reported here were made during acute exacerbations of the disease

The results obtained from examinations of the patients were compared either with those of healthy subjects examined under comparable conditions at the department of clinical physiology during the course of the investigation or with results from healthy subjects reported in the literature Some body parameters of patients and controls are given in Table 1

A characteristic case history which has not been described earlier is the following

Case A 2 (K S)

A female clerk born 1937 who had always had unusually well developed calves At ten years of age she began to experience dyspnoea and tachycardia on exertion When walking her legs tired relatively soon, and the calves became stiff and hard At that time she could

Table I *Some body parameters of the patients compared with male and female control subjects*

Case no	A 2	A 3	A 4	B 1	B 2	C 1	D 2	D 3	E 1	Controls Mean (range) n = 4	n = 5
Symbol in figures	●	■	▲	◐	□	◪	○	■	△		
Sex	♀	♂	[illegible]	♀	♂	♂	[illegible]	♂	♀	♂	♀
Age y	[illegible]4	21	23	34	31	25	27	[illegible]6	43	27 (25–[illegible]8)	23 (19 29)
Height cm	17[illegible]	174	166	166	172	171	165	172	156	179 (167 189)	163 (159 173)
Weight kg	58 6	63 [illegible]	60 8	6[illegible] 6	5 [illegible]	55 5	66	65 3	51 7	70 9 (58 72)	56 6 (50–60)
Pulse rate at BMR test	64–66	56 58	66 [illegible]0	78 88	8[illegible] 92	90 9[illegible]	64–68	56–60	74 74		
Pulse rate at orthostatic test beats min	1[illegible]1	105	117	160	11[illegible]	1[illegible]0	93	94	118		
Hemoglobin conc g 100 ml	12 00	13 55	11 7[illegible]	11.30	13 00	13 3	11 3	13 7	13 4	13 1 (12.[illegible] 13 8)	12.[illegible] (11 6–13 0)
Total amount of hemoglobin (THb) g	[illegible]05	660	465	385	510	640	575	730	425	719 (595 865)	477 (395–575)
Blood volume l	4 [illegible]	4 9	4 0	3 4	3 9	4 9	5 1	5 3	3 [illegible]	5 6 (4 3 6 4)	4 0 (3 4–5 0)
Heart volume ml	6[illegible]0	810	6[illegible]0	600	470	710	5[illegible]0		500		
Physical work capacity (PWC_{170}) kpm min											
Leg work sitting	180	[illegible]00	160	100	100	140	165	300	160	1[illegible]41 (1050–1500)	745 (640–960)
Leg work supine	[illegible]00	180	[illegible]00	130	75	140	180	275	1[illegible]5		
Arm work sitting	100	150		85		130	75	[illegible]00	75	6[illegible]9 (580–690)	405 (335–465)
PWC_{170} predicted from THb cf Fig 3	6[illegible]5	9[illegible]0	550	400	600	880	750	10[illegible]0	450	1009 (811 1[illegible]41)	6[illegible] (491 779)
Basal O_2 uptake (BMR) ml min	[illegible]14	256	[illegible]6	193	[illegible]7	235	[illegible]07	[illegible]8	197		
BMR per cent deviation from predicted	7	9	11	0	11	9	7	[illegible]	13		
Vital capacity l (BTPS)	3 4[illegible]	5 53	3 7	3 [illegible]8	3 90	5 [illegible]5	4 3	5 [illegible]7	3 63	5 89 (5 3 7 [illegible])	3 [illegible]0 (3 –4 4)
Total lung capacity l (BTPS)	4 5	7 37	4 53	4 [illegible]5	5 39	6 8	5 5	6 87	4 69	7 87	4 87
Residual volume total lung capacity	0 [illegible]4	0 [illegible]5	0 19	0 [illegible]4	0 [illegible]7	0 [illegible]3	0 [illegible]1	0 23	0 [illegible]3	0 [illegible]1 (0 15–0 [illegible]5)	0 [illegible]3 (0 16–0 [illegible]9)
Maximal voluntary ventilation l (BTPS) min	[illegible]13	169	100	109	157	[illegible]18	[illegible]4	[illegible]16	112	[illegible]36 ([illegible]11 [illegible]76)	131 (1[illegible] 147)
Forced expiratory volume in one sec l (BTPS)	3 40	5 15	3 07	3 05	3 37	4 36	3 47	4 [illegible]0	3 [illegible]3	5 18 (4 6–5 9)	3 30 ([illegible]80–3 98)
Alveolar gas mixing index %	1 4	1 8	[illegible]	1 5	[illegible]1		1 6	0 9	1 5		

neither run nor ski During the following years, tachycardia occurred even on fairly slight physical exertion and going up hills or stairs caused cramps in the calves At 17 she had a remission that lasted for some years, during which the symptoms occurred only on severe physical exertion This period was however followed by an exacerbation and then she could only walk a few hundred meters without resting In the evenings her ankles were often swollen Even work which only involved the arms soon caused dyspnoea and tachycardia and afterwards the arms felt heavy and weak This period of deterioration lasted a few years after which she felt fairly well She could lead a comparatively normal life as long as she avoided physical exertion The urine had never been dark coloured

The patient was admitted to the Department of Neurology University Hospital of Umeå on April 5 1961

Physical condition The patient was of ordinary body build She had neither resting dyspnoea nor tachycardia A weak systolic murmur was heard over the precordium Blood pressure 140 90 mm Hg

Neurological condition Marked hypertrophy of the calves The largest circumference of the calves was 39 cm The calf muscles were hard but not tender on palpation She had no muscular atrophy or pareses The muscle and abdominal reflexes were normal and equal on both sides Babinski's sign was negative bilaterally Sensibility was normal

Roentgen examination of the lower legs showed hypertrophy of the calf muscles without deposition of fat

The electroencephalogram and electrocardiogram were normal. Laboratory examinations of blood and urine normal. In this case no myoglobin or porphyrines were demonstrated.

METHODS

Most of the examinations were made when the patients stayed at the Department of Neurology, Umeå University Hospital for 2–3 weeks (case B I after a three months stay at this and other hospitals).

Isometric muscle strength for short contractions was determined with ergometers described by Asmussen and Heebøll-Nielsen (2). The force applied on the ergometer was recorded on a potentiometer recorder. Three attempts at producing a maximal force of muscle contraction were made for each of the tested muscle groups and the maximum value was used.

In order to test the endurance of the muscles maximum contractions were performed every 3rd second for 10 min. The force of the contractions was recorded continuously (3) and the pulse rate measured every minute.

Physical working capacity. The patients' physical working capacity for prolonged exercise with large muscle groups was tested on a bicycle ergometer (15) on which cycling in a sitting or supine position as well as arm work (cranking) in the sitting position could be performed. The work tests were made according to Sjöstrand (31) and Wahlund (35) with stepwise increase in work load and 6 min's work on each load. The work loads chosen for the patients were small—usually 100 and [2]00 kpm/min.

The physical working capacity (PWC_{170}) for the different types of work was defined as corresponding to the work load resulting in a pulse rate of 170 beats/min. The determination was based on the fairly linear relation between work load and pulse rate at the 6th minute of work on each load. PWC_{170} was obtained by graphical inter- or extrapolation. No correction was made for non-steady state of pulse rate.

Ventilation and O_2 uptake at rest and during exercise was determined by collecting the expired air in Douglas bags. The volume was measured in a gasometer and the concentrations of O_2 and CO_2 were analyzed in a micro-Scholander apparatus (28).

Mechanical efficiency was calculated by the following formula:

$$\text{mech. eff.} = \frac{100 \times \text{mechanical work performed (kpm/min)}}{4.7\ (\text{total Cal. prod./min—basal Cal. prod./min})}$$

The basal oxygen uptake was calculated according to Harris and Benedict. The caloric coefficient for oxygen was set at 4825 at rest and at 4900 during work (3).

Electrocardiograms were recorded at rest, during an orthostatic test, and during and after exercise.

The methods for recording electrocardiograms and phonocardiograms, for the measurement of total hemoglobin (THb), blood volume and heart volume in the prone position were in principle the same as those used by Holmgren et al. (1[?]).

Lung function studies included determination of lung volumes with a closed helium spirometer system. Maximum voluntary ventilation (MVV), forced expired and inspired vital capacity in one second (FEV_1, FIV_1) were measured with a Bernstein spirometer. The intrapulmonary gas mixing was examined with the N_2 single breath test (10).

Central and peripheral circulation. Usually as the last examination the patients were studied at rest and during exercise with arms or legs after the introduction of several catheters. A heart catheter, usually a double lumen catheter, was placed with the tip in the pulmonary artery and polyethylene catheters were introduced percutaneously according to Seldinger (29) in the brachial artery and in peripheral veins such as the femoral and axillary vein.

In the axillary vein the tip of the catheter was placed a few cm lateral to the thoracic wall as judged by fluoroscopy of the supine subject.

The tip of the catheter in the femoral vein was in most cases about 10 cm distal to the ligamentum inguinale. In some patients a second catheter was introduced into the femoral vein in the proximal direction and its tip was placed just above the ligamentum inguinale. There were negligible differences in oxygen saturation between blood samples taken simultaneously from the two catheters at rest as well as during exercise. In some patients of family A only a proximal catheter was used.

In patient A 4, who was pregnant in the [2]nd to 3rd month, a thin polyethylene catheter was introduced percutaneously without fluoroscopy into the pulmonary artery.

Blood pressures were recorded in the systemic and pulmonary circulation. The zero pressure level was the midaxillary line. The cardiac output was determined according to Fick's direct method. O_2 uptake was measured and blood samples were taken between the 4th and 6th minutes of exercise on each work load.

Blood flow to the legs during exercise was estimated from the femoral arterio-venous oxygen difference and the oxygen uptake at rest and during exercise in principle according to the method described by Donald et al. (8). It was assumed, however, that only 80 [%] of the extra oxygen uptake during exercise was used in the legs. If 100 [%] of the extra oxygen uptake during exercise had been assumed to be used in the legs the calculated blood flow to the legs of some patients would have exceeded the cardiac output. In accordance with Donald et al. it was further assumed that the blood flow through the legs was 0.75 l/min when the resting cardiac output was 4–6 l/min and when the resting cardiac output exceeded 6 l/min the value for leg blood flow was taken as 1.0 l/min. The blood flow to the legs during exercise was then obtained from the equation

$$F_E = \frac{F_R \cdot (\text{a-v } O_2 \text{ diff.})_R + 0.8 \cdot (\dot{V}_{O_2 E} - \dot{V}_{O_2 R})}{(\text{a-v } O_2 \text{ diff.})_E}$$

where F = blood flow to the legs, $\dot{V}_{O_2}$ = oxygen uptake and the subscripts E and R indicate exercise and rest respectively.

Oxygen saturation of blood was determined spectrophotometrically (16) or by the method of van Slyke and

Table II *Isometric muscle strength of some muscle groups in four patients*
The coefficient of variation of the normal values was of the order of 15 to 20

Case	Hand grip[a] kp R	Hand grip[a] kp L	Hand grip[a] % of normal R	Hand grip[a] % of normal L	Elbow flexion[b] kp R	Elbow flexion[b] % of normal R	Forward flexion of trunk kp	Forward flexion of trunk % of normal	Backward flexion of trunk kp	Backward flexion of trunk % of normal
A 2	38	35	91	84	16	87	37	68	—	—
C 1	41	40	73	69	35	109	66	111	44	53
D	9	—	88	—	23	118	33	85	46	79
C 1	19	19	60	60	15	83	24	79	—	—

[a] Normal values from Asmussen and Heebøll Nielsen (2)
[b] Normal values from Ringqvist (34)
R = right, L = left, kp = kilopound

Neill (33). As a rule, van Slyke analyses were used in at least one of the determinations of cardiac output for each patient parallel with the photometric method.

Lactate concentration of the blood was determined according to Strøm's modification (3) of the method described by Barker and Summerson (4). *Pyruvate concentration* of the blood was measured by a method devised by Friedemann and Haugen (11) as modified by Huckabee (18). Blood samples for the determination of lactate and pyruvate were taken simultaneously at the various sampling sites, and precipitation was carried out in direct connection with the sampling.

Excess of lactate (XL) in arterial and venous blood was calculated according to the formula

$$XL = (L_n - L_0) - (P_n - P_0)(L_0/P_0)$$

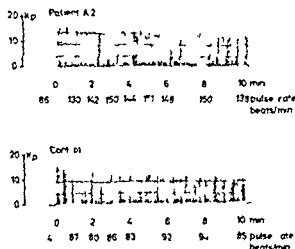


Fig. 1. Isometric muscle strength during repeated maximal contractions of the elbow flexors at a rate of 10/min and simultaneous measurement of pulse rate. Patient A 2 compared with a healthy female control subject. Cf. Table II.

where L_0 and P_0 are the concentrations of lactate and pyruvate at rest and L_n and P_n the corresponding values during exercise (19).

RESULTS

Isometric muscle strength

The general impression from the clinical examination that the muscle strength of the patients was, with few exceptions, normal was confirmed by the measurements of the isometric muscle strength of some muscle groups for short contractions. Table II shows the results of such measurements in four patients compared with normal values. The good muscle function in exercise of short duration is also shown by the normal results of the ventilatory capacity tests (Table I).

The results of repeated maximal contractions of the elbow flexors in patient A 2 and in a female control subject are shown in Fig. 1. The patient's endurance was quite good. The plateau reached after 6–10 min of maximal contractions, was about 70% of the patient's maximal force of contraction. This is somewhat more than the normal average (about 65% at this rate of contractions) which the control subject approached (3). But the patient's pulse rate increased considerably more than that of the control (Fig. 1). A similar result was obtained in patient D 2.

Exercise tolerance

The low capacity of a patient in performing physical work of some duration with large muscle groups is shown by Fig. 2, which demonstrates

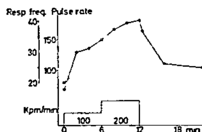


Fig 2 Increase in respiratory frequency (O O) and pulse rate (O – O) during an exercise test (case A 3) The work load was 100 and 00 kpm min

the rapid increase in puls rate during light exer cise on the bicycle ergometer in a typical test It also shows that the pulse rate did not reach a relatively steady state but increased more than normally i e more than 10 beats/min from th 2nd and the 6th minute of work at constant load

When the patients were unable to continue the work they complained of fatigue palpitations and dyspnoea but not particularly of muscle pain or weakness They were still able to move their legs and arms and there were no signs of para lysis Only if they continued the work at a lower intensity for some length of time did muscle pains appear and the muscles might become tense and tender

The low physical working capacity at pulse rate 170 (PWC_{170}) of the individual patients for leg and arm work compared with healthy sub jects is shown in Table I The physical working capacity was also very low in relation to THb and heart volume whereas the pulse rate during an orthostatic test was normal or high (Fig 3)

Mechanical efficiency

The oxygen uptake during exercise was within the normal range or possibly slightly high in relation to the light work The mechanical ef ficiency was th refore within the normal range or possibly slightly low (Fig 4)

Heart function and central circulation

The ECG at rest was normal in s ven out of nine cases In case E 1 and D 2 slight ST and ST T depressions appeared in some ECGs at rest while other ECGs were normal Moderate sympathicotonic ECG changes (13) were ob s rved in the orthostatic test and during exercise in patients B 1 and D 2 In patient E 1 slight ST depressions in praecordial leads corresponding to the left ventricle were present during and after exercise

The heart volume in relation to the total amount of hemoglobin (THb) was within normal limits (Fig 3 C)

Results from the heart cathet rization studies are given in Table III Th blood pressures were normal at rest and during exercise (leg work in the recumbent position) At rest the O saturation was normal in arterial and mixed venous blood as well as the cardiac output and stroke volume During exercise the O saturation of mixed venous blood remained high the a–v O

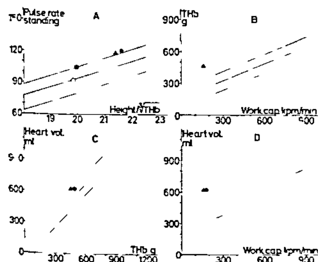


Fig 3 Relations between orthostatic pulse rate physical working capacity and characteristics of body size Symbols as in Table I The physical working capacity for leg work was measured in the sitting position The normal regression lines ± one standard error of estimate were given by Holmgren et al (1?) *A* Pulse rate after 8 min standing, beats min (ordinate) in relation to the index body height (cm) divided by cube root of THb (g) abscissa *B* Total amount of Hb THb g (ordinate) in relation to physical working capacity kpm min (abscissa) *C* Heart volume ml (ordinate) in relation to physical working capacity kpm min (abscissa) *D* Heart volume ml (ordinate) in relation to physical working capacity kpm min (abscissa)

Table II *Isometric muscle strength of some muscle groups in four patients*

The coeffi ient of variation of the normal values was of the order of 15 to 20

	Hand grip[a]				Elbow flexion[b]		Forward flexion[a] of trunk		Backward flexion of trunk	
	kp		% of normal		kp	% of normal	kp	% of normal	kp	% of normal
Case	R	L	R	L	R	R				
A 2	38	35	91	84	16	8[illegible]	3[illegible]	68	—	—
C 1	41	40	7[illegible]	69	35	109	66	111	44	53
D 2	29	—	88	—	[illegible]3	118	33	85	46	79
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[a] Normal values from Asmussen and Heebøll Nielsen (?)
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Neill (33) As a rule van Slyke analyses were used in at least one of the determinations of cardiac output for each patient parallel with the photometric method

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$$XL = (L \quad L) \quad (P \quad P) \quad (L \quad P)$$

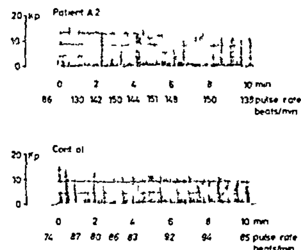


Fig 1 Isometric muscle strength during repeated maximal contractions of the elbow flexors at a rate of 0 min and simultaneous measurement of pulse rate Patient A 2 compared with a healthy female control subject Cf Table II

where L_a and P are the concentrations of lactate and pyruvate at rest and L_e and P_e the corresponding values during exercise (19)

RESULTS

Isometric muscle strength

The general impression from the clinical examination that the muscle strength of the patients was with few exceptions normal was confirmed by the measurements of the isometric muscle strength of some muscle groups for short contractions Table II shows the results of such measurements in four patients compared with normal values The good muscle function in exercise of short *duration is also shown by the normal results of* the ventilatory capacity tests (Table I)

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The low capacity of a patient in performing physical work of some duration with large muscle groups is shown by Fig 2 which demonstrates

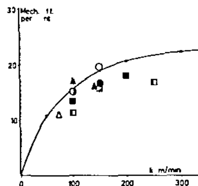


Fig 4 Mechanical efficiency in per cent in relation to work load Symbols as in Table I Normal values from ref (1)

difference low and the cardiac output increased out of proportion to O uptake The stroke volume remained normal The abnormal relations compared to normal conditions appear in Fig 5

Peripheral circulation

Legs At rest the O saturation of the femoral venous blood was normal During exercise on the bicycle ergometer in the supine position the O saturation of the femoral venous blood did not decrease but even increased in some cases (Fig 6 A–C) Estimation of the blood flow through the legs indicated that an abnormally large proportion of the cardiac output particularly with regard to the light work performed passed through the legs (Table IV)

Arms The O saturation of the axillary venous blood was normal at rest but just as in the femoral veins during leg exercise it did not decrease normally during arm exercise on the bicycle ergometer (Fig 7 A–C Table V) On the other hand in a superficial forearm vein it decreased normally

In the same way the oxygen saturation of the femoral venous blood of the resting leg decreased as in controls during arm exercise

Lactate and pyruvate metabolism during exercise

The concentrations of lactate and pyruvate in the blood of the patients at rest were largely normal or slightly high Before the arm work which the patients usually did about 2 hours after a leg work test, it was above the normal range (Table V) During exercise the lactate and pyruvate concentrations rose out of proportion to the work load particularly in the veins of the working extremities (Figs 6 D–I and 7 D–I Tables IV and V) but also in the arterial or mixed venous blood (Tables III–V) The production of lactate and pyruvate in mM/min estimated from the arterio-venous difference and blood flow through the exercising legs was considerably larger than in normal subjects (Table IV) The ratio lactate/pyruvate however decreased during exercise with legs and arms but increased after work, contrary to conditions in normal subjects (Fig 6 G–I and

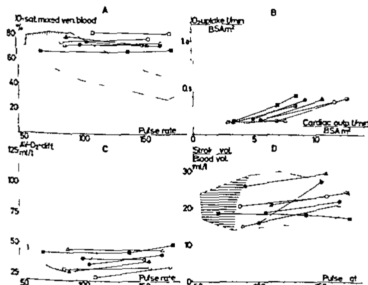


Fig 5 Some characteristic hemodynamic relations of the patients at rest and during exercise Symbols as in Table I The values for each individual case are connected by straight lines The range of normal variation, indicated by thin lines, was obtained from Holmgren et al (14) *A* Oxygen saturation of mixed venous blood per cent, in relation to pulse rate *B* Oxygen uptake divided by body surface area (l/min/m²) in relation to cardiac index (l/min m²) *C* Arterio-venous oxygen difference (ml l) in relation to pulse rate *D* Stroke volume divided by blood volume (ml/l) in relation to pulse rate

Table V *O_2 saturation, lactate and pyruvate concentrations in arterial and axillary venous blood at rest and during arm work (cranking) in the sitting position in patients and controls*

The controls were five male subjects examined during the course of this investigation

	Patients				Controls			
	At rest		Work at high pulse rate		At rest		Work at high pulse rate	
	n	Mean (range)	*n*	Mean (range)	*n*	Mean (range)	*n*	Mean (range)
Work load kpm/min		—	7	114 (75–200)		—	5	5[illegible]0 (400–600)
Pulse rate beats/min	6	95 (74–130)	7	177 (160–198)	5	75 (63–83)		173 (150–195)
O_2 saturation %								
Axillary vein	6	69.4 (56–89)	7	76.3 (63–87)	5	49.7 (43–70)	5	37.9 ([illegible]8–46)
Arterial blood	3	94.0 (89–98)	4	96.8 (94–99)	5	98.1 (96–99)	5	96.6 (94–98)
Lactate concentration mM/l								
Axillary vein	6	[illegible]3 (1.7–3.3)	7	7.9 (5.0–14.7)	5	1.[illegible] (0.8–1.5)	5	7.3 (5.0–10.0)
Arterial blood	3	2.2 (1.2–3.5)	4	6.3 (4.1–8.0)	5	1.1 (0.67–1.5)	5	6.3 (3.9–7.4)
a–v difference	3	0.0 (–0.[illegible]–0.3)		–2.4 (–0.1 – –6.7)	5	–0.05 (–0.[illegible]4–0.04)	5	–1.0 (–1.8–0.79)
Pyruvate concentration mM/l								
Axillary vein	3	0.[illegible]0 (0.18–0.[illegible]3)	[illegible]	0.85 (0.63–1.06)	5	0.1[illegible] (0.08–0.17)	3	0.[illegible]5 (0.[illegible]0–0.31)
Arterial blood	1	0.17	1	0.69	3	0.12 (0.09–0.18)	3	0.[illegible]5 (0.[illegible]1–0.[illegible]9)
a–v difference	1	0.01	1	0.38	3	0.02 (–0.01–0.07)	3	–0.11 (–0.07–0.33)

elbow flexors are engaged. But even such light work may mean a considerable load on the circulation, judging from the marked increase in pulse rate, which did not occur in normal subjects (Fig. 1).

Exercise of some duration with large muscle groups, such as work on a bicycle ergometer, shows the very pronounced functional disturbances of the patients. Tolerance of exercise of this type was poor. Even a low work load of some five to six minutes duration was sufficient to cause hyperventilation and a rise in pulse rate to 170 beats/min or more. Judging by the high pulse rate when the patients were unable to continue the work, the circulatory capacity appeared to be a main factor limiting the patients' tolerance for physical exercise. It was rather general fatigue, palpitations and dyspnoea than weakness or paralysis of the working muscles that seemed to cause the interruption of exercise. The experiments with repeated maximal contractions of the elbow flexors show that the muscles can endure work for a time comparable to that of the work test on the bicycle ergometer.

An analysis of the functional disturbance shows that the oxygen uptake was fairly normal or slightly high in relation to the work load. The mechanical efficiency, calculated from O uptake and external work, was normal or slightly low. Consequently, the gas transport as such should not stress the circulation.

There was no evidence that the heart was insufficient or that the myocardium was affected by the disease. The ECG was normal at rest and during exercise, with occasional exceptions. The heart volume was normal in relation to the total amount of hemoglobin and blood volume. The blood pressures, including the filling pressures of the ventricles, were normal, and so was the stroke volume in relation to blood volume and heart volume. This indicates a normal contractility of the myocardium and a normal pumping action of the heart. The maximal cardiac output measured in the patients during exercise was also of the

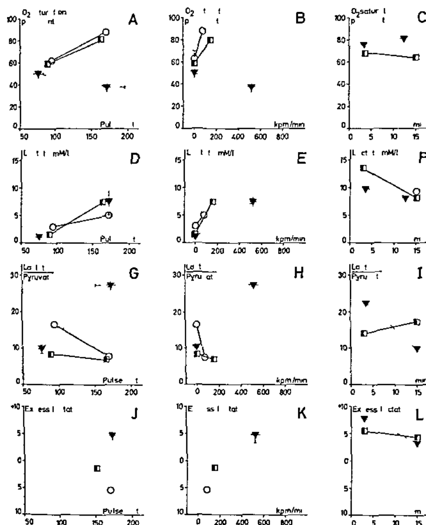


Fig 7 O saturation lactate concentration the lactate/pyruvate ratio and the calculated excess lactate in the axillary venous blood at rest during *arm work* (cranking) on the ergometer in the sitting position and after exercise represented in a similar way as in Fig 6 The patients were compared at rest and during exercise with five healthy controls examined during the course of this investigation After exercise the controls consisted of two subjects (Fig 5 C) and one subject (Fig 5 F I and L)

same magnitude as in healthy subjects during heavy exercise However the patients reached this high cardiac output at a low work load

Evidently the patients required their whole circulatory capacity at a low work load in order to maintain the large blood flow to the exercising limbs At rest the cardiac output and the utilization of oxygen in the peripheral tissues was normal but during exercise the oxygen content of the mixed venous blood was almost the same as at rest and the arterio-venous oxygen difference remained practically unchanged The blood of the axillary and femoral veins during arm and leg exercise respectively also had an abnormally high oxygen saturation A disturbance in the peripheral tissues during exercise

Table VI *Excess lactate of arterial blood (mM/l) in patients and controls during and after leg exercise*

		Exercise			
		At moderate pulse rate	At high pulse rate	3–5 min after exercise	15 min after exercise
Patients	*M*	−1 11	−? 06	−0 0	+5 21
	n	2	3	2	1
Controls	*M*	+0 58	+2 84	+2 05	–
	n	7	10	10	–

Control values from Carlson and Pernow (6)
M = arithmetic mean *n* = no of subjects examined

must be assumed in order to explain this abnormal circulation

It is unlikely that the abnormal circulatory regulation is caused by disturbed nervous vasoregulation as the oxygen saturation decreased in a normal way in the blood of the femoral vein of the resting leg during arm exercise In blood from a superficial vein of an exercising arm which presumably collected blood chiefly from non muscular tissues the blood oxygen saturation also decreased as in the controls (23) This suggests that the reflex regulation of the circulation in resting tissues was normal and that the large blood flow during exercise takes place through the working muscles

It is more likely that abnormal amounts of metabolites in the exercising muscles cause a vasodilatation This assumption is favoured by the fact that the concentrations of lactate and pyruvate in the blood from exercising limbs as well as the calculated lactate and pyruvate production were high for the work performed in comparison with the controls

The pyruvate concentration in blood was remarkably high in relation to that of lactate during exercise This resulted in a low lactate/pyruvate ratio during exercise and a negative excess lactate" production in the myopathy patients with a rapid reversal to positive excess lactate production after exercise

According to Huckabee (20) a negative excess lactate production or excess lactate absorption is common in local tissues but the excess lactate production of the whole body is positive in conditions with increased anaerobic metabolism In normal subjects exercise is known to cause excess lactate accumulation in the body as a whole (21) The excess lactate also increased in the blood from exercising legs (6) and arms (as can be calculated from Table V) of normal subjects The abnormal behavior of the patients during exercise in these respects should according to Huckabee s concept be compatible with shifts in the LDH system and indicate a metabolic disorder

It is not unlikely that the capacity for pyruvate oxidation is lower than normal and insufficient during exercise Such a condition might explain the abnormal lactate/pyruvate ratio during exercise which became normal after exercise when the metabolic demands decreased

Further support for a pathologic muscle metabolism has been obtained from enzyme studies on muscle biopsies Preliminary experiments indicate that the rate of oxidation of pyruvate by isolated mitochondria is slow as compared with controls (9)

So far only the blood concentration of the metabolites lactate and pyruvate have been examined It seems probable that parallel to the increased formation of lactate and pyruvate abnormal amounts of other metabolites are also transferred Presumably they cause vasodilatation and a blood flow through the muscles out of proportion to oxygen uptake (hyperkinetic circulation)

Factors other than circulation which may limit the exercise tolerance do not seem to have been critical Muscle function has been discussed previously The lung function was normal and the patients hyperventilated during exercise The oxygen saturation of the arterial blood was normal at rest and during exercise It therefore seems justified to conclude that a main factor limiting exercise tolerance in these patients is the circulatory capacity which is fully utilized even for light work because of the pronounced vasodilatation in exercising muscles

One patient with an exertional paroxysmal myoglobinuria described by Kontos et al (22) who in some respects resembled our patients did not have such a marked hyperkinetic circulation during exercise and the a v O difference increased considerably more During leg exercise comparable to that performed by our patients there was no appreciable change in the carbon dioxide tension and pH of arterial blood and consequently no change in the bicarbonate concentration Therefore the lactate and pyruvate production must have been smaller than in our cases This patient also had a larger than normal excess lactate production during exercise in contrast to our patients

The patients with McArdle s disease described by Schmid and Mahler who did not produce lactate and pyruvate during exercise were also opposite in type to our cases especially with regard to lactate and pyruvate metabolism (26 27) There are also other muscular metabolic disturbances in which the production of lactate is small even during ischemic exercise (25)

A hyperkinetic circulation (high output state)

is found in several diseases such as anaemia hyperthyreoidism beri beri cirrhosis of the liver systemic arterio-venous shunts vasoregulatory asthenia Paget s disease of the bone etc (34) Patients with these diseases have a high cardiac output at rest and therefore differ from the myopathy patients who had a hyperkinetic circulation only during exercise The myopathy patients had normal BMR liver function tests and Hb-concentration and several of the previously mentioned diseases can therefore be excluded

Patients with vasoregulatory asthenia (12) have been examined with methods similar to those applied for the myopathy patients In the two conditions the physical working capacity was low and the lactate and pyruvate production was high at a low work load (7 17) One difference was that the myopathy patients had a hyperkinetic circulation only during exercise At rest the circulation to the limbs was normal judging from the normal O saturation in the femoral and axillary veins whereas in the patients with vasoregulatory asthenia the O saturation was high both at rest and during exercise However the O saturation of the femoral venous blood during exercise decreased more in the patients with vasoregulatory asthenia (mean O saturation 42 at heart rate 160–185 beats/min) (7) than in the myopathy patients Further the pyruvate production was high in relation to that of lactate in the myopathy patients which resulted in a negative excess lactate production while the patients with vasoregulatory asthenia had an excess lactate production during exercise

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EFFECTS OF HYPERVENTILATION ON ECG IN PATIENTS WITH CIRCULATORY DISTURBANCES

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Abstract The ECG has been recorded in patients with vasoregulatory asthenia, coronary insufficiency and arterial hypertension as well as in healthy controls at rest, after hyperventilation during an orthostatic test and a work test

S T and T depressions usually of sympathicotonic type appeared or were accentuated after hyperventilation, particularly in patients with vasoregulatory asthenia who also showed similar changes during an orthostatic test. Such ECG changes were uncommon in the other groups

The results sustain the concept that an increased sympathetic autonomic nervous tone provoked by hyperventilation causes the S–T and T depressions particularly in patients with vasoregulatory asthenia

The diagnostic value of the hyperventilation ECG for analysing functional S–T and T depressions is emphasized as well as the diagnostic difficulties in the evaluation of ECG changes in patients who hyperventilate for instance during a hypoxia test.

It is known that hyperventilation may cause S–T and T depressions of the electrocardiogram (16 17) It has been suggested that ECG changes occurring in patients with anxiety pain and fever as well as after smoking and physical exercise may be caused by hyperventilation (cf 17) There are different opinions as to the mechanism by which hyperventilation causes these ECG changes Respiratory alkalosis (9 18 26) autonomic reflexes (16 29) coronary vasoconstriction and changes in the position of the heart (21) have been assumed to be possible causative factors

For several years ECGs after hyperventilation were recorded as a routine in patients examined for diagnostic purposes with ECG at rest during an orthostatic test and during and after a work test Our impression was that ECG changes after hyperventilation were found frequently in patients with functional or sympathicotonic S–T and T changes in the orthostatic test and particularly in those with the vasoregulatory asthenia syndrome (12 13) These observations initiated this investigation on the frequency of ECG changes occurring after hyperventilation in some groups of patients with certain types of circulatory disturbances and in healthy controls

MATERIAL

All patients, who were examined by means of an ECG in connection with a work test on a bicycle ergometer at the Department of Cl nical Physiology during a two year period (about 4000) performed a hyperventilation and an orthostatic test From these certain groups of patients described below and a number of healthy control subjects examined during the same period were selected for the investigation The groups of patients included all subjects of those examined who fulfilled the criteria defined below A survey of some anthropometric and other data for these groups is given in Table I The patient groups are in the main included in those described by Borg and Linderholm (5)

Patients w th vasoregul to y asthenia (Group VA) This group consisted of 11 males and 8 females 20–50 years of age It included all patients in whom the physical working capacity (W ₁₇₀) was low i e deviated more than 1 S D from the regression line between THb and W and HV and W ₁₇₀ of normal subjects given by Holmgren et al (12) In this group W ₁₇₀ increased towards normal values in relation to total hemoglobin and heart volume after ganglionic blocking with chlorisondamine (Ecolid) about 0 1 mg kg subcutaneously and in some cases also after physical training (2)

The VA patients usually had a high pulse rate at rest after 10 min in the supine position (mean 97 beats/min for males and 107 for females) and during an orthostatic test (mean 121 beats min for males and 1_5 for females)

Heart catheterization was performed in three of these cases They were found to have a hyperkinetic circulation a finding that completed the VA-syndrome All VA patients had normal arterial blood pressures and no signs

Table I *Some anthropometric and other data of the groups examined Mean ± S D*

Group	Sex	n	Age (y)	Height (cm)	Weight (kg)	Blood pressure mm Hg: At rest Syst	At rest Diast	Highest work load syst	Pulse rate beats/min: At rest	Standing	Hypervent	Highest work load	Work intensity kpm/min: W_{130}	W_{170}	W_{max}	HV (ml)	THb (g)
VA	c	8	32	162	59	141	86	175[a]	107	125	139	182	185	319	39?	537	468
			±9 3	+3 6	+6 0	+11 3	+9 5	14 5	+18 8	18 8	+17 0	±6 5	+91	+86	±135	±96	±51
	ɔ	11	8	175	66	147	80	185[b]	97	1?1	119	187	375	560	700	70?	658
			±8 1	+5 3	+10 0	+16 3	+ 8 1	17 3	+19 7	+ 1 ?	4 7	+8 2	+160	+111	±141	±135	±80
N_I		8	21	166	56	? 4	74	193	76	89	94	183	5?5	7 0	792	516	484
			+1 5	+5 9	+5 3	±7 3	+9 4	+13 4	+13 4	+17 2	+17 8	±3 9	+69	±103	±67	±43	±69
	ɔ	11	23	179	69	139	80	03	68	88	10?	182	879	111	1 50	734	693
			+3 4	+6 5	+8 6	10 7	+9 3	+ 0 5	±14 7	+10 1	+21 7	±6 6	+132	±125	±.04	±168	±105
C		11	56	163	66	147	90	198[b]	74	89	86	155	501	—	473	—	—
			±9 0	+5 9	+7 7	+15 0	+5 7	+18 7	+13 3	16 4	+14 3	±1?6	+190	—	±177	—	—
	♂	28	57	173	74	145	9?	190	77	86	89	141	744	—	580	—	—
			+7 1	+5 3	+8 2	+1?2	+7 8	±?6 9	±1 0	±14 8	+18 0	±14 5	±213	—	±?37	—	—
H		?7	53	161	67	04	121	75	76	90	90	158	465	—	498	—	—
			±6 1	+6 0	+11 4	±17 4	+15 1	+17 3	±11 7	±15 1	±13 8	±15 8	±131	—	±155	—	—
	♂	18	58	175	81	193	119	274	77	87	88	153	781	—	803	—	—
			±7 0	+6 0	+11 3	+ 8 0	±13 7	±18 6	±17 9	±?1 3	±19 1	±16 0	±161	—	± 32	—	—
N_{II}		8	5	161	73	144	88	0[b]	68	85	84	152	669	801	650	—	—
			±5 8	+5 9	+12 3	±9 9	±8 5	±30 5	±10 8	±1? 3	±10 8	±4 3	+74	±105	±67	—	—
	♂	10	53	176	79	134	90	08[a]	72	86	83	16?	885	1091	955	—	—
			±6 4	±4 2	±10 6	+6 6	±11 2	±13 3	±7 9	+11 5	±10 4	±8 8	±116	±157	±76	—	—

[a] n – ? [b] n 3 n 4

of hyperthyroidism or anemia Most of the VA patients desribed by Arvedson et al (?) are included in this group

Patients with arterial hypertension (Group H) This group consisted of 45 patients 18 males and ?7 females over 45 years of age When referred to the Department for an ECG examination their diagnosis was hypertension Their arterial blood pressure at rest was 160 mm Hg or more systolic and 95 mm Hg or more diastolic During the work test their systolic blood pressure was 40 mm Hg or more The ECG changes during or after the work test did not suggest coronary insufficiency

Patients with coronary insufficiency (Group C) This group consisted of 39 patients 28 males and 11 females They were as a rule referred to the Department for an ECG examination during exercise to sustain the clinical diagnosis of angina pectoris They had ECG changes during and after exercise typical of coronary insufficiency (10 25) During exercise they suffered anginal pains Their arterial blood pressure was below the limits stated for the group with arterial hypertension

All patients with valvular heart disease ECG or history suggesting an earlier myocardial infarction with digitalis therapy and patients unable to perform hyperventilation properly were excluded from Groups VA H and C

Control group N_Y consisted of 19 subjects 11 males and 8 females, 0–30 years old, who were compared with the VA patients. They were healthy volunteers who had normal relationships between W_{170}, heart volume and THb They had a normal ECG at rest, during and after exercise

Control group N_I consisted of 18 subjects 10 males and 8 females over 45 years of age who were sent to the Department for a work test with ECG for various reasons (preoperative check up health control etc) They had a normal ECG at rest and an ordinary physical working capacity i.e the W_{170} was 900 kpm min or more for males and 600 kpm min or more for females The ECG was usually normal during and after exercise or showed slight and unspecific S–T depressions which did not indicate coronary insufficiency The subjects did not complain of angina pectoris during exercise They had blood pressures below the limits given for the hypertensive patients.

METHODS

Recording of ECG ECGs with lead I II III aVR, aVL, aVF and precordial leads CR CR, CR CR and CR were recorded with an ECG apparatus Mingograph 4 (Elema Schonander) at rest during an orthostatic test after hyperventilation and during and after an exercise test During exercise the indifferent electrode was on the forehead (H) and the CH leads 2, 4 5 and 7 were used.

At rest ECGs were recorded after 10 min rest in the supine position

Orthostatic test The subject stood for 8 min leaning with the back of his head against a wall after which the ECG was recorded

The *hyperventilation test* was performed in the supine position after the orthostatic test and when any ECG changes which might have occurred during this test had disappeared The subject was encouraged to ventilate maximally for 30 sec Immediately after the hyperventilation the ECG was recorded. In some subjects the expired gas was collected in order to check its magnitude In most cases the hyperventilation was observed by experienced test leaders Most subjects performed a satisfactory hyperventilation. In case of poor cooperation the test was interrupted and no ECG was recorded

Work test The subject worked sitting on a bicycle ergometer (14) and the test was performed according to Sjostrand (2) and Wahlund (8) During exercise ECG were recorded after 2, 4 and 6 min on each work load and immediately 4 and 10 min after the end of the exercise

The physical working capacity at pulse rate 150 and 170 (W_{150} and W_{170}) was obtained by inter or extra polation, making use of the almost linear relationship between pulse rate and work load

Total haemoglobin of the body (THb) and blood volume were measured by the alveolar CO method (3) with some modifications (cf 2) A small amount of CO was added to the rebreathing system and the CO analyse were made by the palladium molybdenum tube method (1)

Heart volume (HV) was determined in the prone position (for ref cf ?)

Arterial blood pressure was measured according to Riva–Rocci and the diastolic blood pressure was taken as the pressure measured when the sound disappeared (4)

Evaluation of ECG changes S–T and T changes at rest, after hyperventilation during the orthostatic test and during and after the work test were evaluated according to a four grade scale described by Holmgren et al (13) This scale was originally used to evaluate the degree of sympathicotonic ECG changes but has also been found useful for classifying S–T and T changes of other types found in the examined patient groups

Statistical methods In the statistical calculations a change in an ECG compared with the ECG at rest was considered to have occurred when in connection with hyperventilation an orthostatic test or a work test, the ECG was classified one or several degrees higher according to the 4-grade scale The statistical significance of differences in frequency of changes after hyperventilation, during the orthostatic test etc in the different groups has been estimated by means of the Chi method

RESULTS

The frequency and type of ECG changes at rest and the ECG changes that appear after hyperventilation during the orthostatic test and during and after the work test varied in the different groups The frequency and degree of S–T and T changes according to the four grade scale (see Methods) are given in Table II

Table 1 *Some anthropometric and other data of the groups examined Mean* ± S D

Group	Sex	n	Age (y)	Height (cm)	Weight (kg)	Blood pressure mm Hg: At rest Syst	Blood pressure mm Hg: At rest Diast	Blood pressure mm Hg: Highest work load syst	Pulse rate beats/min: At rest	Pulse rate beats/min: Standing	Pulse rate beats/min: Hypervent	Pulse rate beats/min: Highest work load	Work intensity kpm/min: W_{150}	Work intensity kpm/min: W_{170}	Work intensity kpm/min: W_{max}	HV (ml)	THb (g)
VA	♀	8	32	162	59	141	86	175	107	125	139	182	185	319	392	537	468
			±9 3	±3 6	±6 0	±11 3	±9 5	±14 5	±18 8	±18 8	±17 0	±6 5	±91	±86	±135	±96	±51
	♂	11	28	175	66	147	80	185[b]	97	121	119	187	375	560	700	702	658
			±8 1	±5 3	±10 0	±16 3	±28 1	±17 3	±19 7	±21 2	±24 7	±8 2	±160	±111	±141	±135	±90
N_I	♀	8	21	166	56	124	74	193	76	89	94	183	525	720	792	516	484
			±1 5	±5 9	±5 3	±7 3	±9 4	±13 4	±13 4	±17 2	±17 8	±3 9	±69	±103	±67	±43	±69
	♂	11	23	179	69	139	80	03	68	88	102	182	879	1112	1250	734	693
			±3 4	±6 5	±8 6	+10 7	+9 3	±20 5	±14 7	±10 1	±21 7	±6 6	±132	±125	±204	±168	±105
C	♀	11	56	163	66	147	90	198[b]	74	89	86	155	501	—	473	—	—
			±9 0	±5 9	±7 7	±15 0	±5 7	±18 7	±13 3	±16 4	±14 3	±12 6	±190	—	±177	—	—
	♂	28	57	173	74	145	92	190[c]	77	86	89	141	744	—	580	—	—
			±7 1	±5 3	±8 2	±12 2	±7 8	± 6 9	±12 0	±14 8	±18 0	±14 5	±213	—	±237	—	—
H	♀	27	53	161	67	204	121	275	76	90	90	158	465	—	498	—	—
			±6 1	±6 0	±11 4	±17 4	±15 1	±17 3	±11 7	±15 1	±13 8	±15 8	±131	—	±155	—	—
	♂	18	58	175	81	193	119	274	77	87	88	153	781	—	803	—	—
			±7 0	±6 0	±11 3	±28 0	±13 7	±18 6	±17 9	±21 3	±19 1	±16 0	±161	—	±232	—	—
N_{II}	♀	8	52	161	73	144	88	202[b]	68	85	84	152	669	801	650	—	—
			±5 8	±5 9	±12 3	±9 9	±8 5	±30 5	±10 8	±12 3	±10 8	±4 3	±74	±105	±67	—	—
	♂	10	53	176	79	134	90	208	72	86	83	162	885	1091	955	—	—
			±6 4	±4 2	±10 6	±6 6	±11 2	±13 3	±7 9	±11 5	±10 4	±8 8	±116	±157	±76	—	—

[a] n – [illegible] [b] n – 3 [c] n – 4

Table IV shows how comparatively well the S–T and T changes agree in the various groups after hyperventilation and during the orthostatic test There are no systematic differences between the ECG changes in these two tests

In the VA patients the ECG changes increased less during exercise than during the orthostatic test and after hyperventilation

The *localization* of the ECG changes that appeared after hyperventilation is shown in Table V The most common changes were in leads II III and CR_{4-7} In some patients changes appeared in leads CR_1 The different localization of the ECG changes had no relation to the direction of the mean QRS or T vectors nor to the type of ECG changes at rest in the VA group

Group H

In the group with arterial hypertension 33 patients of 45 had S–T and T depressions *at rest* of varying degree and of a type commonly seen in arterial hypertension The ECG changes at rest were more marked among the 25 patients with hypertension and a low physical working capacity than among the hypertensives with an ordinary working capacity i e those male and female patients who worked for 6 min on a work load of at least 900 and 600 kpm/min respectively

On *hyperventilation* just as in th standing position and during exercise ECG changes appeared or increased in some patients (Table II III and Fig 3) Of the 45 hypertensive patients seven showed ECG changes after hyperventilation of a higher degree than at rest according to the four grade scale Next to the VA group ECG changes after hyperventilation were most common in the group with arterial hypertension The frequency of these changes seemed to be independent of whether the physical working capacity of the patients was low or ordinary During the *orthostatic test* seven hypertensive patients showed increased ECG changes Five of them showed increased changes on hyperventilation

Just as in the case of the VA patients the ECG changes after hyperventilation and during the orthostatic test were on an average of the same degree (Table IV) During exercise ECG changes appeared or increased quite frequently as commonly seen in patients with arterial hypertension

The localization of the ECG changes which appeared or increased on hyperventilation is shown by Table V In 3 out of 7 patients there were changes in lead I (Fig 3) a change which seemed to be more common among th hypertensive patients than in the other groups examined In all seven cases ECG changes appeared in the left precordial leads As in the VA group there was no relation between the different localizations of the ECG changes and the direction of mean QRS or T vectors or type of ECG changes at rest

Table III *Number of patients in whom the ECG was unaltered or showed an increase or decrease according to the four grade scale after hyperventilation and during the orthostatic test when compared with the ECG at rest*

	Standing at rest					Hyperventilation at rest					
	−1	±0	+1	+2	+3	−1	±0	+1	+2	+3	+4
VA		7	9	3			7	6	5		1
N_I		17	2				17	2			
H		38	7				38	7			
C	1	36	2			2	36	1			
N_{II}		17	1				17	1			

Table IV *Difference in degree of S–T and T changes according to the four grade scale after hyperventilation compared with those in the orthostatic test*

Column −1 shows the number of patients who had 1 grade more ECG changes in the orthostatic test than after hyperventilation and columns +1 to +3 those who had less marked changes

Hyperventilation standing	−1	±0	+1	+2	+3
VA	3	11	3	1	1
N_I	2	15	2		
H	2	41	2		
C	2	37			
N_{II}	1	16	1		

Table V *The localization of the ECG changes which appeared or increased on hyperventilation*

No of subjects with ECG changes			Localization of ECG changes							
VA	H	N	I	II	III	CR	CR_1	CR	CR	CR
7	3	2		+	+					+
1	2	1								
2	1									+
1	1									+
1				+						+

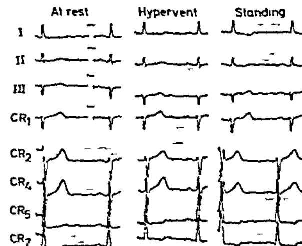


Fig 3 ECG changes typical of patients with hypertension at rest after hyperventilation and during an orhostatic test.

Group C

In 32 out of 39 patients with coronary insufficiency there were ECG changes of various degrees *at rest* Slight S–T and T depressions (Table II) were more common and more pronounced among the 26 patients with low physical working capacity in those with an ordinary physical working pacity

After *hyperventilation* one patient with a low physical working capacity had slightly increased S–T depressions in CR_4 In two patients the ECG changes decreased slightly

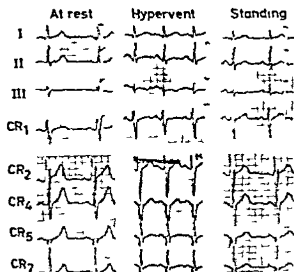


Fig 4 ECG changes appearing after hyperventilation and during an orthostatic test in a healthy control subject.

During the *orthostatic test* ECG changes increased slightly in two patients one of whom also showed increased S–T depressions on hyperventilation They also decreased slightly in one case in the same way as after hyperventilation

During exercise the S–T and T depressions typical of coronary insufficiency appeared in all cases according to the principles of selection of the patients of this group

Control groups N_I and N_{II}

In the control groups the ECG was normal at rest in all subjects In three subjects slight ECG changes (grade 1) appeared during the orthostatic test after hyperventilation or during exercise see also Fig 4 and Table II

DISCUSSION

ECG changes after hyperventilation are known to be fairly common (16 17) Hyperventilation is frequent in patients with anxiety pain and fever as well as after smoking and physical exercise *ECG changes seen in such subjects have been* attributed to hyperventilation There are several theories about the mechanism causing the S–T and T depressions provoked by hyperventilation

A respiratory alkalosis was previously assumed to contribute to the ECG changes (9 18 26) Later investigations have shown that a pH change does not affect the ECG (19) nor does a decreased CO_2 tension as assumed by some authors (9 18 26) seem to be important Thus breathing of up to 11 per cent CO_2 gas mixtures does not prevent T inversions provoked by short periods of hyperventilation corresponding to those used in our investigation Thompson (26) concluded in 1943 that neither the degree of alkaline shift nor the extent of lowering of the CO_2 combining power is the sole factor in determining the kind and magnitude of the S–T and T changes

Changes of the position of the heart in connection with hyperventilation (21) and coronary heart disease (26) have also been assumed to cause ECG changes after hyperventilation

Later investigations have shown that hyperventilation increases the cardiac output (3 7 27) and increases muscle blood flow (6) These hemodynamic changes and most probably also the ECG changes are likely to be caused by changes in the balance of the autonomic nervous system

It has been assumed that an increased vagal tone caused the S–T and T depressions (29) A relatively increased sympathetic tone however seems to be a more likely cause ECG changes after hyperventilation are common in anxious and tense subjects (29) who are often known to have an increased urinary excretion of catecholamines (10) In psychiatric patients ECG changes occurring after hyperventilation were abolished by the adrenergic beta receptor blocking agent propranolol (11)

Our results lend further support to the assumption that S–T and T depressions after hyperventilation are caused by an increased sympathetic tone They show that ECG changes which appear or are added to those of the ECG at rest are common after hyperventilation in patients who show ECG changes of functional or sympathicotonic type (13) at rest and/or have signs of a vasoregulatory disturbance (vasoregulatory asthenia) (12) The vasoregulatory asthenia syndrome which is characterized by symptoms such as a high pulse rate at rest and particularly during an orthostatic test and a low physical working capacity ($W_{1\ 0}$) in relation to heart volume and THb seems to be associated with an increased sympathetic tone (2) The investigation also shows that patients with vasoregulatory asthenia who have S–T and T depressions during an orthostatic test commonly exhibit ECG changes also after hyperventilation ECG changes appearing after hyperventilation correlated positively with similar changes during an orthostatic test ECG changes of this sympathicotonic type are comparatively unusual in patients with arterial hypertension and coronary insufficiency as they are in healthy controls

It is interesting that the S–T and T changes after hyperventilation and during the orthostatic test seem to be relatively more common in hypertensives than in the groups with coronary heart disease and the controls The hypertensives are also to some extent similar to the VA patients in their rating of perceived exertion in relation to pulse rate (5)

Recording of ECGs after hyperventilation seems to be diagnostically valuable for the analysis of functional or sympathicotonic S–T and T changes In this respect it may be regarded as a complement or as an alternative procedure to the recording of an ECG during an orthostatic test The hyperventilation ECG has the advantage of being easily and rapidly performed also in bedridden patients

There are reasons for caution in evaluating ECG changes in patients who hyperventilate It is likely that the falsely positive hypoxia ECGs are due to the hyperventilation involved in this diagnostic test (9)

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RELAPSING POLYCHONDRITIS

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Abst act A case of relapsing polychondritis in a 27 year-old man is reported There was involvement of the ear nasal and costal cartilage He had periodical pains in the spinal column the elbow hip- and knee joints Biopsy of the nasal septum showed perichondritis and moderate chondritis. Ele ated ESR, patholo ic electrophoresis of serum anemia and a transient elevation of the white blood count were recorded. There was an abundance of eye symptoms scleroiritis, sclerokeratitis of a special type and exudative chorioretinitis Treatment alternatively with acetylsalicylic acid, corticosteroids and monophenylbutazon was effective Local and general treatment with corticosteroid had a good effect on the eye symptoms.

In 1923 Jaksch Wartenhorst (8) reported on the first patient with relapsing polychondritis Dolan et al (5) described two patients with the same disease and analyzed forty nine other cases from the literature A few cases were reported (1 3 4 14) after this review The first publication from Denmark (9 10 11) appeared in 1962 and from Norway in 1963 (6) The terminology is vari able and several of the names used are mentioned by Goldwater (6) Pearson et al (13) introduced the term relapsing polychondritis which is the one usually used at present.

The illness is characterized by painfully in flamed ears nose trachea larynx costochondral junctions and peripheral joints Cartilage inflam mation usually predominates but episcleritis iritis hearing impairment cataracts anemia abnormal ities of liver function myocarditis and aortic valvular insufficiency have all been noted as ancillary features of this syndrome (5) The symp toms may be of long duration or appear inter mittently with recurrences

This report concerns a typical case of relapsing polychondritis which is especially interesting due to the abundance of eye symptoms

CASE REPORT

The patient was a 27 year-old man without previous disease but he had a trauma against the nose in early 1964

Preceded by an attack of fever he had during the period September to December 1964 transient edema and rubor in the cartilage of the left ear and septum nasi The symptoms continued for three to four weeks in each area, and he developed flopping of the ear and a per manent saddle-shaped dorsum nasi (Figs. 1 and 2) The patient was treated by his doctor with antibiotics He also had pains located on both sides of the sternum which increased during forced inspiration For a short time he had light pains in the left elbow region

On admission to the Medical Department VII Ullevål Hospital the septum nasi was thick and red, and there were pains corresponding to the insertion of the four distal costae to the sternum, both on the right and left s de His temperature was 38 3 C ESR was 94 mm/1 h later 1?7 mm/1 h Hemoglobin 1?9 g/100 ml The Hb later decreased to 10.5 g/100 ml. MCH 33 and 27 respecti ely The maximum WBC was 16 600 per mm but after a week it became normal with a normal dif ferential count. The platelet count was normal

B opsy from septum nasi (Fig 3) showed moderate chondritis, and the perichondrium was infiltrated with lymphocytes and plasma cells in many places A piece of tissue was covered w th stratified epithelium with cornification and there were moderate chronic changes of inflammation in stroma. The tissue was stained with PAS and alcian blue Metachromasia was observed in the cartilage but not more than normal Nor was there any increase in neutral and acid mucopolysaccharides in the surrounding tissue The d agnosis was chronic peri chondritis and moderate chondritis

LE-cells were not observed and immunoelectrophoresis was normal Electrophoresis of serum showed a sl ghtly lowered albumin and γ-globulin fraction, increased α_2-globul n fraction and a small increase of β-globulin Blood cultures, antistreptolysin titer antistaphylolysin t ter gonococcalcomplement fixation test WR and thy roid antigens were negative ECG was normal.

X ray examination of the total sp nal column il osacral joints, left elbow thorax and ear cartilage showed normal find ngs. There was calcification in the laryngeal cartilage uncommon in his age along with a residuum of nasal



Fig 1 Deformities of nose

Fig 2 Deformities of left ear



Fig 3 Biopsy of nasal cartilage

fracture However the deformity of his nose was also localized to the nasal cartilage

Saccharide chromatography in several samples of urine showed an unusual pattern However most of the changes were caused by salicylate and further investiga tions were not performed Acid mucopolysaccharides could not be observed in the urine after staining with alcian blue

Ninety six hour urine specimens were examined using a modified CPC technique (cetylpyridinium chloride) (2 7) 400 ml distilled water and 100 ml cetylpyridinium chloride were added to one liter of urine Before adding cetylpyridinium chloride the pH was adjusted to 7 No hexosamine was found in the supernatant after centri fugation The precipitate was converted to sodium salt



Fig 4 Infiltrates in the corneal stroma and episcleritis

and analyzed. It contained less than 4 of hexosamine Further fractionations were therefore not possible but most probably there were not increased amounts of mucopolysaccharides in the urine

The patient developed eye symptoms (Figs 4–6) about one year later and was hospitalized in the Department of Ophthalmology Ullevål Hospital He had scleroiritis once and sclerokeratitis of a special type four times There was edema of the cornea and many discoid infiltrates in the corneal stroma. He also had bilateral uveitis with exudative chorioretinitis The inflammation improved upon local treatment with solutions of atropine and chloramphenicol but oculoguttae cortisoni had the best effect Peroral treatment with prednisolone was also used. A more complete description of the eye symptoms will be found in another publication (3)

During the first period in the medical department he was treated with acetyl salicylic acid with relatively good effect later during a period with high fever he received methylprednisolone The temperature returned to normal. Treatment with monophenylbutazon also had a good effect. He had to use analgetic tablets and chlorpromazine for tranquilizing.

At times he had pains in the lumbal part of the spinal column hip-joints and left knee joint.

Later the γ fraction of serum protein increased to a normal value and about one year after the disease had started the serum electrophoresis and ESR were quite normal The patient began working eighteen months after the appearance of his first symptoms

DISCUSSION

The usual diagnostic signs of relapsing polychondritis are recurrent inflammation of two or more cartilaginous areas (5) In the 14 patients reported by Kaye and Sones (12) three or more



Fig 5 Exudative chorioretinitis in acute stage

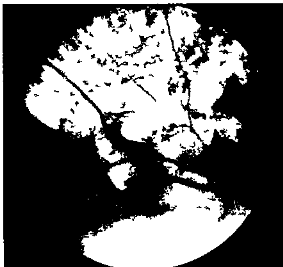


Fig 6 The chorioretinitis two weeks later inactive stage scarring and pigmentations

cartilaginous sites were involved in all but three In the review by Dolan et al (5) ear cartilage and nasal inflammation were the most common manifestations in 88 and 82 % of the cases respectively In our case ear nasal and costochondral cartilage elbow hip- and knee joint and joints in the spinal column were affected Other usual symptoms (5) have been fever and laryngotracheal involvement Episcleritis and/or conjunctivitis was found in 60% of the cases in the latter report iritis in 27%. Especially interesting are the many eye symptoms observed in our patient The type of exudative chorioretinitis as far as may be seen from the literature has been observed once in the earlier cases of relapsing polychondritis (1) The average life span from onset of symptoms is 7 1 years But there is a wide spectrum of duration from ten months to twenty four years and perhaps longer In the review by Dolan et al (5) there were six respiratory deaths

In our patient biopsy from the nasal septum showed perichondritis and moderate chondritis Increase of neutral or acid mucopolysaccharides in the tissue could not be observed nor were there acid polysaccharides in the urine after staining with alcian blue A 96-hour urine collection did not show increased amounts of mucopolysaccharides in the urine Tsaltas (16) found that increased excretion of chondroitin sulphate in the urine had not been recorded except in animal

experiments But Kaye and Sones (12) found an increase of the urinary acid mucopolysaccharides during exacerbation of the disease in three of their patients During remission this test was nor mal in a fourth case The results of these in vestigations in our patient may therefore depend upon the period in which they were performed

Serum electrophoresis showed as in many re ported cases hypoalbuminemia There was also hypo γ globulinemia in the first period There were increased fractions of α and β globulin Serum protein abnormalities have been reported (5) in 52 % In more than half of the published cases there was an anemia that has never been characterized (5) As in our case the anemia as usual is slightly hypochromic While it is gen erally held that relapsing polychondritis is an autoimmune disease further investigations con cerning the etiology are being made Intravenous injection of crude papain into rabbits produced cartilaginous dissolution of the ears within 4 hours (15) It is demonstrated that papain produced a loss of chondroitin sulphate from the cartilaginous matrix Evidently the primary defect in patients results in acidophilic staining of the cartilage re presenting a loss of matrix acid mucopolysac charides (17)

Hypervitaminosis A also produced cartilaginous lesions similar to those from papain (15)

Immunofluorescent studies (5) have shown greater binding of conjugated test serum to normal human cartilage than serum from controls sug gesting an immune mechanism in the pathogenesis of the disease

Acetyl salicylic acid corticosteroids and mono phenylbutazon were used alone in different pe riods supplemented by analgetic and tranquilizing medication There was an effect of all the three drugs perhaps best with monophenylbutazon Dolan et al (5) are of the opinion that administra tion of corticosteroids seems to be the treatment of choice but salicylates alone are reported (12) to have some effect on fever and chondritis

General and local treatment with corticosteroids had an excellent effect on the eye symptoms in our case

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SPIROMETRIC STUDIES OF VENTILATORY CAPACITY IN ELDERLY PEOPLE

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Abstract Dynamic lung function tests have been studied in apparently healthy men and women who in essential respects may be considered as representative of the total population within the age range concerned. The results are compared with some previously reported normal values Certain not unimportant differences exist in spite of essentially identical method It seems probable that these differences are due mainly to differences in the composition of the series compared in previous investigations the selection of subjects does not appear to have been done on an adequate statistical basis

In clinical work the need for measurements both of static lung volumes and of the ventilatory utilization of certain of these volumes has increased greatly Evaluation of the data obtained obviously requires the availability of comparative or normal values A number of such values are to be found in the literature Most frequently cited is probably the American investigation from 1961 The Veterans Administration Army Cooperative Study of Pulmonary Function reported by Kory et al (6) In Scandinavia data from a study in Goteborg in 1963 have been published concerning the lung volumes and ventilatory capacity in healthy persons of ages 7–70 years These values at least in Sweden and the rest of Scandinavia have been used as a basis for calculation of normal values both in clinical routine work and in clinical research

It is evident however that in previous studies the subjects investigated have been selected according to certain principles e g taken from certain occupational categories Normal values obtained in this way therefore do not agree with certainty with those of the total population The aim of the present investigation was to study the lung volumes and ventilatory capacity in a material which could be reasonably regarded as representative of the total population within the age groups concerned

MATERIAL

Linder et al. (7) performed in 1961 a comprehensive health investigation in Uppsala on a statistically selected and representative section of the total population, of ages 21–70 years The total group consists of 135 men and 179 women aged 52–66 years from the 1961 investigation Of these 65 men and 65 women were selected at random for inclusion in the investigation (the 130 who were last called to the health examination) Four men and two women refused to take part five men and two women were excluded since they were found at the examination to have diseases of the types exemplified below Thus 56 men and 61 women remained and were already in 1961 selected to comprise the material of the present investigation This latter series did not differ from the total group with regard to social group physical effort requirements in their daily work body height, body weight hemoglobin value or sedimentation rate However compared with the total group" there was some difference with regard to general state of health as assessed by the examining doctor Thus from the selected group" those individuals were excluded who had any diseases involving reduced pulmonary or cardiac function or changes in cardiac function which might have been expected to affect the ventilatory capacity and lung volumes Examples of diseases which were represented amongst the total group but not the selected group" are organic cardiac disease pulmonary tuberculosis and rheumatoid arthritis

For the present investigation the subjects were divided, according to the 1961 data, into three age groups 52–56 years (1 men and 23 women) 57–61 years (21 men and 25 women) and 62–66 years (14 men and 13 women) The majority of these individuals were subjected to a longitudinal five year follow up study It was thus possible to obtain data for a fourth age group viz. 67–71 years, which was judged to be of interest although this group cannot be immediately compared with the other groups as the time of examination differs

Table I *Physical characteristics of the material*

Group	Examination year	Sex	No	Age y Mean	Age y Range	Height cm Mean	Height cm S D	Height cm Range	Weight kg Mean	Weight kg S D	Weight kg Range
52–56 I	1961	♂	21	54 0	51–56	174 2	6 1	164–183	72 4	9 3	57 0–87 0
		♀	23	54 2	52–56	163 6	5 5	153 173	67 6	9 8	50 0–88 0
57–61 II	1961	♂	21	59 6	57–61	175 6	6 7	164 190	73 4	9 1	56 4–90 0
		♀	25	58 7	57–61	164 0	6 5	151–178	66 7	8 5	50 [illegible]–89 2
62–66 III	1961	♂	14	63 5	62–66	174 6	7 6	164 193	68 6	10 2	54 0–86 5
		♀	13	63 8	62–66	160 1	6 7	148–170	64 3	11 0	50 0–91 4
67–71 IV	1966	♂	10	68 7	67–71	174 3	8 5	164 193	70 8	9 9	54 0–87 0
		♀	10	69 0	68–71	161 0	7 5	147 170	63 7	11 6	49 0–85 0

The lung function values reported for the latter age group were obtained in 1966 the subjects (10 men and 10 women) concerned belonging to the group of subjects who in 1961 were 62–66 years

Table I gives certain anthropometric data distributed by age and sex As mentioned above these data agreed with those of the total population taking into consideration age and sex.

None of the subjects included in this investigation had a history of chronic heart or lung disease or symptoms of any disease which may have influenced the cardiopulmonary function, and all had been apparently healthy as regards heart and lungs during the six month period immediately preceding the investigation In no case did physical examination chest X ray or electrocardiography reveal definite signs of cardiopulmonary disease However no person was excluded from the material because of arterial hypertension because of the well known difficulties in establishing a clinically significant boundary between pathological and normal in this respect The diastolic pressure was 110 mm Hg or more in three subjects their values being 120 115 and 110 mm Hg. None of these three subjects showed any clinical, roentgenological or electrocardiographic signs of left ventricular hypertrophy In no case was anything pathological noted in the ocular fundi, e g exudation or haemorrhage

All subjects were considered to be fully capable mentally of cooperating in the different stages of the investigation

METHOD

The light weight spirometer used (model Spirokombi, Kifa Ltd. Stockholm) was a slightly modified version (1) of that described by Bernstein et al (2) The procedures for lung volume determination and ventilatory capacity were essentially as described by Berglund et al (1) Birath et al. (3) and Grimby and Soderholm (4) The international nomenclature of lung volumes and tests was used. In the determination of vital capacity (VC) forced expiratory volume in one second (FEV_1) and maximum voluntary ventilation with a fixed respiratory frequency of 40 per minute (MVV_{40}) or with a respiratory frequency chosen by the patient (MVV) every individual performed each test at least three times By FEV $_{\%}$ is meant FEV $_{1}$ expressed as a percentage of VC or FVC (forced vital capacity) whichever was the largest FEV_1 was estimated at the point of the spirogram occurring 0 10 second after the first expiratory deflection whether the curve immediately assumed its steepest slope or showed an initial part of flow acceleration. For measuring maximum expiratory flow (MEF) a peak flowmeter (11) was used.

Table II *Mean value standard deviation and standard error of the mean for the various function tests after grouping with regard to sex and age*

	VC l			$FEV_{1.0}$ l			FEV %			MVV_{40} l/min			MVV_F l/min			MEF l min		
	M	S.D	S.E	M	S D	S E	M	S D	S E	M	S D	S E	M	S D	S E	M	S D	S E
Males																		
52–56 I	4 54	0 91	0 20	3 45	0 79	0 18	74 3	8 2	1 9	108 4	[illegible]2 7	5 1	142 9	33 2	7 4	514 0	70 3	15 7
57–61 II	4 [illegible]7	0 71	0 16	3 03	0 57	0 13	69 7	23 6	5 1	99 3	22 5	4 9	119 9	33 1	7 2	515 7	52 3	11 4
62–66 III	4 06	0 51	0 13	2 97	0 61	0 16	71 4	12 1	3 2	93 8	24 4	8 1	108 7	24 5	6 5	457 9	65 3	17 5
67–71 IV	3 93	0 47	0 15	2.86	0 41	0 13	68 8	8 4	2 6	85 0	18 1	5 7	106 4	14 5	4 6	482 0	76 3	[illegible]4 1
Females																		
52–56 I	3 05	0 44	0 09	2 40	0 37	0 08	81 2	4 9	1 0	76 0	10 6	2 [illegible]	96 7	16 9	3 5	4[illegible]9 1	55 7	11 6
57–61 II	2 95	0 42	0 08	2 20	0 35	0 07	76 5	6 9	1 4	68 5	12 0	[illegible] 4	83 6	15 2	3 0	3[illegible]0 8	50 8	10 2
62–66 III	2.83	0 47	0 13	2 25	0 50	0 14	79 8	4 1	1 2	69 8	15 2	4 2	85 2	13 5	3 7	389 2	67 1	18 6
67–71 IV	2.87	0 42	0 13	2 14	0 38	0 1[illegible]	73 6	5 6	1 8	69 0	14 9	4 7	85 6	17 5	5 5	386 5	66 5	21 0

Table III *Mean value standard deviation and standard error of the mean for the difference between the highest and second highest value for the various function tests at the investigation in 1961*

The difference expressed in absolute values

	VC l			FEV1 0 l			MVV40 l/min			MVVF l/min			MEF l/min		
	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE
Males															
52 56 I	0 17	0 15	0 03	0 13	0 10	0 02	4 90	3 75	0 82	8 50	6 56	1 43	23 80	18 77	4 10
57–61 II	0 16	0 15	0 03	0 14	0 12	0 03	5 24	3 91	0 85	9 00	6 86	1 50	20 00	16 62	3 63
62–66 III	0 17	0 15	0 04	0 07	0 07	0 02	5 38	4 08	1 13	7 50	5 48	1 46	19 30	15 47	4 13
Females															
5 –56 I	0 13	0 11	0 02	0 12	0 11	0 02	4 35	3 38	0 71	6 57	5 30	1 10	23 48	21 06	4 39
57–61 II	0 16	0 13	0 03	0 10	0 10	0 02	4 88	3 63	0 73	6 9?	5 34	1 07	17 20	15 56	3 11
62–66 III	0 16	0 13	0 03	0 13	0 11	0 03	5 46	4 07	1 13	6 00	4 62	1 28	20 00	15 93	4 42

Before the investigation began the subject was carefully instructed and was allowed an initial trial run All spirometers were operated by a trained nurse All volumes are given at body temperature and ambient pressure saturated (BTPS) The volumes at BTPS were obtained by multiplying the volumes at ATPS by an average factor of 1 10 (actual range 1 08–1 12) The ranges of room temperature and barometric pressure in these experiments were 18–25 degrees and 730–780 mm Hg respectively

The difference between duplicate checks was analysed by calculating the difference between the highest and the second highest values for each test For these differences see Tables III and IV

RESULTS

Table II shows the mean value standard deviation and standard error of the mean for vital capacity and certain functions measuring ventilatory capacity distributed by sex and age groups It can be seen in the table that for all functions there is on the whole a linear relationship between the different age groups The table shows further that the standard deviations are relatively large i e the scatter within the age groups is pronounced With the exception of maximal expiratory flow where it usually is about 15 the standard deviation is of the order of 20 %

The differences between the highest and second highest values for each test are given in Table III (absolute values) and Table IV (relative values) As seen in these tables the differences are relatively small being on an average 3–4 % of the maximum values This indicates a good reproducibility of the determinations at immediate repetition When individual maximum ventilation values in 1961 and 1966 were compared for the group examined longitudinally relatively small individual differences were found as will be reported in detail in a forthcoming paper an average

Table IV *Mean value standard deviation and standard error of the mean for the difference—expressed in per cent—between the highest and second highest values for the various function tests at the investigation in 1961*

	Diff VC			Diff FEV1 0			Diff MVV			Diff MVV			Diff MEF		
	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE
Males															
5 –56 I	3 89	3 51	0 77	3 96	3 35	0 75	4 66	3 52	0 77	6 09	4 63	1 01	4 57	3 56	0 78
57–61 II	4 08	3 63	0 79	4 65	3 80	0 85	5 31	3 99	0 87	7 67	5 80	1 27	3 84	3 17	0 69
62–66 III	3 73	3 13	0 84	2 69	2 55	0 68	6 42	4 93	1 37	7 23	5 36	1 43	4 3	3 40	0 91
Females															
5? 56 I	4 28	3 90	0 83	5 49	4 69	0 98	5 96	4 71	0 98	6 94	5 63	1 17	5 18	4 56	0 95
57–61 II	5 44	4 4	0 88	4 86	4 82	0 98	6 78	5 10	1 0	8 08	6 13	1 2	4 37	3 91	0 78
6?–66 III	6 ?9	5 28	1 46	5 77	4 56	1 26	8 17	6 18	1 71	6 94	5 4?	1 50	5 24	4 09	1 13

Table V *Found values expressed in per cent of predicted values according to investigations referred to in the text*

	VC l			FEV1 0 l			FEV			MVV40 l/min			MVVF l/min			MEF l/min		
	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE
Males																		
52–56 I	90 0	12 7	2 9	96 5	17 5	4 0	105 1	12 1	2 8	88 2	19 8	4 4	94 5	20 2	4 5	103 8	13 3	3 0
57–61 II	85 3	13 9	3 0	90 2	15 5	3 5	107 2	17 8	4 0	87 4	19 4	4 2	84 0	23 2	5 1	115 9	13 5	3 0
62–66 III	83 9	11 2	3 0	91 4	21 1	5 6	107 6	18 2	4 9	86 1	26 2	7 3	80 0	17 7	4 7	107 3	16 1	4 3
67–71 IV	87 1	12 2	3 8	94 0	19 3	6 1	105 7	14 5	4 6	84 8	17 9	5 7	82 5	18 2	5 8	118 1	20 6	6 5
Females																		
52–56 I	91 8	11 1	2 4	95 7	9 4	2 0	103 6	9 5	2 0	87 3	10 4	2 2	88 6	17 0	3 7	118 9	12 4	2 6
57–61 II	89 0	12 1	2 5	88 7	11 4	2 3	99 9	11 1	2 3	81 3	12 7	2 5	85 3	18 0	4 1	109 3	11 3	2 3
62–66 III	92 0	11 7	3 3	97 8	19 0	5 4	105 6	11 1	3 1	87 3	16 5	4 6	85 4	17 9	5 0	111 9	18 1	5 0
67–71 IV	95 7	10 0	3 2	98 3	13 8	4 4	104 2	10 1	3 2	88 9	19 1	6 0	90 5	18 5	5 9	111 0	18 3	5 8

decrease of about 5% was observed with a standard deviation of the individual differences of 8–10%

From the data reported for the Goteborg series values were predicted for each of our subjects for the different tests In Table V a summary is given of the results obtained from which it is seen that on an average the values for our sub jects deviate considerably fom the predicted aver age This difference is noted throughout for all ge groups and almost all functions With regard to MEF our subjects showed on an average a significantly higher value than the comparative values which however were not obtained from Sweden but mainly from a study in England (8 11) On the other hand our values are lower than those reported from Oslo by Vale (10) in spite of the fact that the frequency of respiratory symptoms in Norway (9) appears to be higher than in Sweden The differences between our values and those of Vale are difficult to evaluate but it is not improbable that principles used in the selection of subjects constitute a factor of im portance Vale s subjects were a selection of those persons who had undergone routine X ray lung examination

The relationship between different ventilatory capacity tests and age body height body weight can be seen in Table VI

In the multiple regression analyses different

Table VI *Regression equations and residual standard deviations (R S D) for each determination of ventilatory capacity*

		Sex	Regression coefficients Age y	Height cm	Weight kg	Constant	R S D
VC		♂	(− 0 032 ± 0 0?0)	+ 0 046 ± 0 013	+ 0 021 ± 0 010	− 3 515	0 6
		♀	− 0 0 9 ± 0 013	+ 0 034 ± 0 008	(− 0 003 ± 0 005)	− 0 757	0 38
FVC	*	♂	− 0 043 ± 0 020	+ 0 046 ± 0 013	+ 0 0?4 ± 0 009	− 3 380	0 61
		♀	− 0 049 ± 0 014	+ 0 031 ± 0 009	(− 0 002 ± 0 006)	+ 0 740	0 40
FEV1 0		o	− 0 045 ± 0 020	(+ 0 019 ± 0 013)	+ 0 021 ± 0 010	+ 0 898	0 61
		♀	− 0 032 ± 0 01˛	+ 0 0˛3 ± 0 009	(+ 0 001 ± 0 005)	+ 0 252	0 36
FEV	*	♂	(− 0 271 ± 0 306)	− 0 474 ± 0 205	+ 0 331 ± 0 151	+ 147 063	9 3
		♀	(− 0 441 ± 0 280)	(+ 0 087 ± 0 196)	(− 0 031 ± 0 119)	+ 91 748	8 3
MVVF		♂	− 3 055 ± 0 920	(+ 1 181 ± 0 612)	+ 1 014 ± 0 441	+ 23 633	28 4
	*	♀	− 1 360 ± 0 511	+ 0 824 ± 0 337	(+ 0 312 ± 0 217)	+ 12 899	15 1
MVV40		♂	− 2 101 ± 0 858	(+ 0 444 ± 0 571)	(+ 0 601 ± 0 411)	+ 100 662	26 4
		♀	(− 0 605 ± 0 378)	+ 0 632 ± 0 249	(+ 0 274 ± 0 160)	− 14 143	11 2
MEF	*	♂	− 4 500 ± 1 969	(− 1 539 ± 1 310)	(+ 1 662 ± 0 944)	+ 374 408	60 7
	*	♀	− 4 306 ± 1 757	+ 2 637 ± 1 157	(+ 1 012 ± 0 744)	+ 15? 028	51 9

The significance level of the regression equation is denoted by asterisks = $P < 0\ 05$ * − $P < 0\ 01$
Insignificant regression coefficients have been put within brackets

types of functions have been tested For each type of function the regression coefficients and the standard errors were calculated together with the residual standard deviation The significance of the function types was tested not only by determination of the standard error of estimate but also by estimating the coefficient of determination In all analyses except FEV % for women the regression analyses appeared to give a significant explanation of the variation in the dependent variable For FEV o in men and MVV_{40} in women however this significance was not very strong (> 0 05) In some cases (VC FVC $FEV_{1\,0}$, MEF in men and FEV % MVV_{40} in women) the adding of the weight variable to the regression equation did not considerably increase the significance of the explanation of the variation in the dependent variables The adding of the weight variable should increase the explanatory capacity of the regression if the weight variable in itself has some explanation to give and if the weight variable is not too strongly correlated to the other explanatory variables The correlation between weight and height is somewhat greater for men ($r = 0\ 36089$) than for women ($r = 0\ 29316$)

When the interaction term $d \times$ age $\times$ height was added to the equation $y = a + b \times$ age $+ c \times$ height, we found that the regression coefficient for this third variable was significant in the following tests in men, viz FVC $FEV_{1\,0}$ MEF MVV_F and MVV_{40} and in no test in women. Equations including the interaction term in every test, as expected showed somewhat higher explanatory capacity than equations without this term but as a rule lower explanatory capacity than equations including the weight as a third variable As can be seen from Table VI the regression coefficient for weight was never very high and, in most tests in which it was significant was of little importance

All the computations involved were performed by Uppsala Datacentral University of Uppsala, with Mr Adam Taube D Ph Lecturer in Statistics University of Uppsala as statistical adviser

DISCUSSION

It was evident that for the majority of the tests which constituted a measure of ventilatory capacity and where comparison with the Göteborg values was possible our subjects showed, on an average lower values On the whole the same was found on comparison with the values reported from the USA by Kory et al (6) The following discussion will however be concerned mainly with the comparison with the Goteborg series for two main reasons firstly in principle the same method was used for both investigations and secondly both series comprised healthy Swedish individuals No normal values for maximal expiratory flow were reported from the Goteborg study however and our values for this test were therefore compared with those obtained by other authors

In principle there would seem to be two possible reasons for the fact that our values were on an average somewhat lower than those for the Goteborg series (*a*) differences in composition of the two series and (*b*) discrepancies arising in the method e g less cooperation from the subjects in one investigation than in the other or differences in calibration of the apparatus

The principles for the selection of subjects in the Göteborg investigation and in our study differ in some essential points In our study we aimed at a statistical selection from the total population from which persons were excluded with diseases resulting in reduced cardiac or pulmonary function or in changes in cardiac function which might influence the lung volumes and ventilation capacity while the Göteborg material was selected in such a way that it cannot be regarded as representative of a total population In the Goteborg study the male subjects were selected from six occupational categories representing both sedentary and physical workers these included a group of naval officers The women consisted mainly of housewives belonging to different organizations and a small number were office workers or telephonists who had taken part in a health investigation It seems reasonable to assume that the differences in selection of the subjects may well explain the discrepancies in the results

As mentioned above the differences could also have been due to differences in cooperation of the subjects of the two studies It would seem possible that the somewhat lower average ventilatory capacity in our subjects may have been due to less cooperation on their part but this is contradicted by the relatively small average dif

ferences between the highest and second highe t values achieved in the different tests Even if these latter differences were often somewhat larger than the corresponding differences in the Gote borg study there was no great discrepancy

The subjects of our study underwent the sam tests for ventilatory capacity after an interval of five years It was found that for each subject these later results showed good agreement with those obtained five years earlier taking into con sideration the average functional reduction that was observed with higher age on the later oc casion This longitudinal follow up study is re ported in a separate paper

In the light of these results i e the good re producibility at the first investigation and also the good agreement between this and a later in vestigation under similar conditions it seems rea sonable to assume that the cooperation of our subjects was satisfactory

Further it is improbable that the apparatus was faulty it was checked intermittently and showed correct calibration on all occasions

Although our subjects showed on an average a lower ventilation capacity than the Goteborg series their average maximum expiratory flow was relatively high On comparison with the normal values reported by Wright et al and Lockhart et al it was found that the values of our subjects—as a group—were significantly higher This applied to both sexes and appeared to be independent of age within the range covered by this study Here too the difference can prob ably be explained by differences in the composi tion of the material predicted values having been calculated from results obtained in England Be tween England and Sweden however there is a large difference as regards the prevalence in older age groups of lung disease with obstructive re duction of the ventilation capacity In the Upp sala district only about 5% of the total popula tion in the age groups concerned (5) fulfil the criteria for bronchial asthma and chronic bron chitis as established by the American Thoracic Society while the figure for chronic bronchitis only obtained in England according to Mork (9) is significantly higher This difference in th prevalence of obstructive lung disease may be the reason for the higher mean values for maximal expiratory flow in our series

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PHYSICAL WORK CAPACITY AND STATIC LUNG VOLUMES IN ELDERLY PEOPLE

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Abst act Results of determinations of lung volumes and physical work capacity in apparently healthy individuals of ages 57–71 years are reported. Function parameters for physical work capacity are correlated to certain lung volumes and parameters constituting measures of ven tilatory capacity The series would appear to fulfil to a reasonable degree the requ rement of being representati e of the population w thin the age range concerned which does not seem to have been taken into account to the same extent in previous invest gations. In this study as in pre ious investigations, it is found that the maximal achievement capacity for circulation limited work (W_{max}) decreased with increasing age but in addition our study in contrast to previous investigations, ind cates a decrease in submaximal indirect measures of physical work capacity (W_{130}, W_{150}, W_{180}) with increasing age Th s should mean that the reduction in physical work capacity with in creasing age is not only due to a decrease in the maximal heart rate (HR_{max}) but that other factors are also of importance It is probable that a reduced degree of physical training plays an important role in this respect

No strong correlations are found either between lung volumes and physical work capacity or between ventila tion capacity and physical work capacity In this respect our results differ from the results of previously published studies on younger persons. The hypothesis is considered that the regression of physiological function with increas ing age may ha e different time courses for the respira tory and the circulatory systems

In a previous paper we published normal values for elderly apparently healthy persons for dif ferent tests measuring the ventilation capacity The aim of the present investigation was to deter mine on largely the same subjects, normal values for physical work capacity and for static lung volumes and further to correlate certain func tions of physical work capacity to certain lung volumes or quotients between lung volumes and also to certain parameters constituting a measure of ventilation capacity

MATERIAL

The subjects of the present study were included in the series upon wh ch we studied the ventilatory capacity in the year 1961 The principles for the selection of subjects —who as a group could be regarded as representative of the total population within the a e group concerned—have been described in a prev ous paper The investiga tions reported here concerning physical work capacity and lung volumes were performed in 1966 and therefore the fall-out of a certain number of subjects was un avoidable Reasons for the fall-out were death (4 subjects) illness which rendered the examination impossible (6 subjects, 4 of whom with psychiatric disorders) re fusal to take part (3 subjects) change of place of res idence or unattainable (3 subjects) or the fact that the respiratory or cardiac functions had become clearly re duced as a result of a disease which had become mani fest during the f ve year period (3 subjects) In the exam inat on of physical work capacity there was, further a fall-out of five subjects the reason in four of them being illness (disorders of the lower extremities) and in one of them refusal to take part.

Lung volume determinations were performed on 97 of the 98 subjects Phys cal work capacity tests were per formed on 84 subjects in 1966 The classification of these subjects into sex and age groups is given below For reasons of illness, nine subjects who took part in the work tests are not included in the results of deter minations of physical work capacity the reasons for these exclusions were arterial hypertonia with a diastolic blood pressure exceeding 110 mm Hg (4 subjects) card o-scleros s and coronary insufficiency (4 subjects) inter mittent claudication (1 subject) ischial lumbago (1 subject)

The subjects of the present in estigation were divided into the following age groups 57–61 years (?0 men, ?1 women) 6 –66 years (18 men 21 women) and 67–71 years (10 men 10 women) Since all of them were sub jected to a longitudinal fi e year follow-up study it was possible to obtain certain data also for a fourth age group viz 5?–56 years (?0 men, 21 women) This latter group comprised the subjects who in 1966 belonged to the a e group 57–61 years in this paper data for this group are reported only in Table II i.e the table which

Table I *Physical characteristics of the series*

Age group	Examina tion year	Sex	No	Age y mean	Height cm			Weight kg		
					Mean	S D	Range	Mean	S D	Range
52–56 I	1961	♂	21	54 0	174 2	6 1	164–183	72 4	9 3	57 0–87 0
		♀	23	54 2	163 6	5 5	153–173	67 6	9 8	50 0–88 0
57–61 II	1966	♂	18	59 2	174 3	6 2	164–183	74 2	10 8	53 0–99 2
		♀	21	59.5	163 5	5 7	154–172	67 3	9 3	53 0–91 0
62–66 III	1966	♂	18	65 0	174 3	6 6	165–182	71 8	9 5	55 8–89 0
		♀	20	64 0	163 9	6 8	154–177	68 0	9 0	49 0–81 8
67–71 IV	1966	♂	10	69 1	174 2	8 5	164–193	69 5	9 9	54 0–87 0
		♀	10	69 0	160 6	7 5	147–170	63 2	11 6	49 0–85 0

gives values for physical work capacity none of the other results include observations on this age group This table also shows the distribution into sex and age groups for the subjects included in the study of physical work capacity

METHODS

Electrocardiograms were recorded at rest in the supine position after eight minutes of passive standing, during exercise in the sitting position and after exercise in the supine position The following leads were used I II III aVR aVL aVF V V V V and V During exercise the indifferent electrode was moved to the forehead. The ECG apparatus used was a Mingograf 42 (Elema Schonander Ltd Stockholm)

The physical work test (11 12) was performed on an electrically braked bicycle ergometer (9) The heart rate and respiratory frequency were counted and the ECG was recorded continuously during stepwise increases of work load For the women the test was started as a rule at 200 kpm/min and for the men at 300 kpm/min Each work period lasted for 6 min and the work was increased in steps of 200 and 300 kpm/min respectively until a heart rate of approximately 170 beats/min was reached or until because of some abnormal signs or symptoms, the work test was discontinued. The physical work capacity is arbitrarily defined as the absolute work load performed during steady state at each of the heart rates 170 150 and 130 beats/min (W_{170}, W_{150} and W_{130}) steady state is defined either as 10 beats/min or less change of heart rate from the second to the sixth minute of work, or as 2 beats/min or less change from the fourth to the sixth min If steady state was not reached, the values for W_{170} etc were calculated nevertheless but are specially indicated in the tables and figures The value of work capacity was obtained by numerical extra or interpola tion using the approximately linear relationship between heart rate and work load extrapolation was not per formed for more than 20 beats/min. The highest work load actually performed by the subject for at least 5 min is designated W_{m}

The total lung capacity (TLC) and its subdivisions were determined by the helium dilution method using a closed spirometer (model Spirokombi, Kifa Ltd Stock holm) In contrast to the other spirometer tests how ever this investigation was performed only once on each subject The error of measurement of functional residual capacity for this method has been given by Holmgren (7) as 89 ml and by Grimby and Soderholm (6) as 75°

Table II *Mean value standard deviation and standard error of the mean for physical work capacity after grouping with regard to sex and age*

Age group	W_{130}				W_{150}				$W_{\ 0}$				W_{ma}			
	n	M	S D	S E	*n*	M	S D	S E	*n*	M	S D	S E.	*n*	M	S D	S E
Males																
52–56 I	20	730 5	159 8	35 7	20	924 5	156 8	35 1	19	1129 5	182 4	41 8	20	1014 3	200 7	43 8
57–61 II	18	613 9	129 0	30 4	18	800 6	135 5	31 9	12	990 4	128 0	36 9	18	883 3	161 8	38 1
62–66 III	12	584 6	156 0	43 3	12	755 1	182 6	50 6	7	830 0	184 2	75 2	14	721 4	180 5	48 ?
67–71 IV	10	532 0	97 7	30 9	10	709 3	145 5	48 5	6	812 5	192 0	78 4	10	670 0	170 3	53 9
Females																
52–56 I	21	439 0	128 2	28 0	16	57? 4	136 7	33 2	12	695 0	151 0	43 6	21	571 4	158 6	34 6
57–61 II	17	343 1	133 0	33 3	17	489 4	143 4	35 8	9	588 0	199 1	63 0	18	497 2	151 9	35 8
62–66 III	17	318 8	135 8	33 9	11	389 1	148 7	44 8	9	527 8	153 2	51 1	18	400 0	118 8	28 0
67–71 IV	6	240 0	88 1	36 0	6	360 0	121 2	49 5	2	440 0	56 6	40 0	6	366 7	81 6	33 3

Table III *Regression equations and residual standard deviations (R S D) for each determination of physical work capacity*

The significance level of the regression equations is denoted by asterisks 0 01 < $p \leq$ 0 05 0 001 < $p \leq$ 0 01 0 001 > p

	Sex	Regression coefficients			Constant	R S D
		Age y	Height cm	Weight kg		
W_{130}	♂	−7 75 ± 4 83	1 17 ± 3 37	2 18 ± 2 25	714 2	130 17
	♀	−10 94 ± 6 67	0 40 ± 3 34	0 6 ± 2 55	1110 0?	131 72
W_{150}	♂	−8 37 ± 5 88	−0 86 ± 4 07	3 94 ± 2 63	1158 69	150 53
	♀	−13 48 ± 7 22	−0 16 ± 3 89	2 01 ± 2 91	1167 42	141 54
$W_{?}$	♂	−19 55 ± 6 77	0 45 ± 4 82	5 7 ± 3 06	1658 68	144 12
	♀	−15 49 ± 11 11	1 23 ± 6 34	2 09 ± 4 85	1149 83	167 69
W_{max}	♂	−21 4 ± 5 98	0 ?6 ± 4 17	2 4 ± 2 79	1926 17	161 ?6
	♀	−16 49 ± 5 54	−0 17 ± 2.82	0 84 ± 2.18	1473 13	109 27

RESULTS

The work load at a pulse rate of 130 beats/min (W_{130}) could be calculated for 60 of the 62 men and for 61 of the 63 women W_{150} could be calculated for 60 men and 50 women i e for 97 of the men and 79 % of the women included in the series The corresponding figures for W_{170} were 44 men (71) and 32 women (51 %)

The reasons for discontinuation of the work test varied In five cases the test was discontinued because of a pathological ECG reaction in 22 cases because the pulse rate exceeded 170 beats/min and in 17 cases because the respiratory frequency exceeded 35/min In one subject the reason was severe cramp of the gastrocnemius muscle and in two subjects the test was discontinued for precautionary reasons because of suspected coronary insufficiency in the case history In the remaining subjects the reasons were various subjective symptoms such as general exhaustion and muscular articular pain combined with a relatively high pulse rate

It is evident from the tables that with increasing average age fewer calculations could be made for the work load at a given pulse rate per minute Thus for the men in the two higher age groups and following the given principles W_{150} could be calculated for only 13 of the 24 subjects i e 59 % of those for whom W_{130} could be calculated For the two lower age groups the corresponding figures were 31 of 38 i e 82 % For the women W_{170} could be calculated for only 11 of the 24 in the two higher age groups i e 48 % of those for whom W_{130} could be calculated For the two lower age groups the corresponding figures were 21 of 39 i e 55

The mean work load for the men at a pulse rate of 170 beats/min was 1129 5 kpm/min in the age group 52–56 years 990 4 kpm/min in the age group 57–61 years 830 kpm/min in the age group 62–66 years and 812 5 kpm/min in the age group 67–71 years The corresponding mean values for the women in these age groups were 695 0 588 0 527 8 and 440 0 kpm/min Thus for W_{170} a lower mean value was obtained in the higher age groups than in the lower both for men and women This was also true throughout for W_{130} W_{150} and W_m The different measures of physical work capacity showed no marked relationship either with body height or body weight

Table III gives regression co fficients and equations for W_{130} W_{150} W_{170} and W_m For the two latter functions the equations were significant with the exception of the W_{170} equation for women This equation as well—as also those for W_{130} and W_{150}—would probably have reached the significance level if when programming the regressions consideration had been paid to the mutual correlation between body height and body weight This mutual correlation may mean that the age factor was ascribed less importance than if no such correlation had existed

Table IV shows values for lung volumes and relations between such volumes for both men and women grouped according to age No statistically significant difference between age groups is apparent for any of the volumes studied Table VI gives regression coefficients and equations for

Table IV *Mean value standard deviation and standard error of the mean for the various lung volume tests after grouping with regard to sex and age*

	VC 1			RV 1			FRC 1			TLC 1			RV/TLC			FRC/TLC		
Age group	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE
Males																		
57–61 II	4 20	1 30	0 31	1 96	0 45	0 11	2 97	0 83	0 20	6 03	1 14	0 27	32 54	4 97	1 17	49 15	9 57	2 26
62–66 III	4 30	1 30	0 16	2 46	0 42	0 10	3 50	0 72	0 17	6 50	0 86	0 20	37 82	4 73	1 11	53 82	6 44	1 57
67–71 IV	3 90	0 47	0 15	2 24	0 60	0 19	3 54	0 80	0 25	6 20	0 87	0 27	36 06	6 13	1 94	56 74	7 47	2 35
Females																		
57–61 II	3 00	0 35	0 08	1 49	0 32	0 07	1 99	0 48	0 11	4 35	0 69	0 15	33 73	3 56	0 78	45 76	6 34	1 38
62–66 III	2 90	0 33	0 07	1 81	0 3[illegible]	0 07	2 32	0 46	0 10	4 56	0 59	0 13	39 46	5 09	1 14	50 63	5 94	1 33
67–71 IV	2 80	0 42	0 13	1 74	0 44	0 14	2 32	0 63	0 20	4 48	0 72	0 23	38 85	6 39	2 02	51 15	9 00	2 85

lung volumes and certain relations between volumes All of these equations are statistically significant The nomograms in Figs 1 and 2 illustrate the importance of body weight for FRC and FRC/TLC the two functions for which body weight was found to have the greatest importance in our series

In a previous paper (2) we presented data concerning ventilatory capacity These values were obtained from determinations carried out in the year 1961 The results given in Table V were obtained in the main from the same series of subjects but from determinations made in the year 1966 It was considered justifiable to give these data here since in this paper we report correlation coefficients for the relation between ventilatory capacity and a) lung volumes and b) physical work capacity which latter functions are represented by values from the year 1966 Table VII shows correlations between functions of physical work capacity and lung volumes As can be seen only one pair of the correlations attains significance and then only at a low level The relationships between measures of physical work capacity and ventilatory capacity (Table VIII) are also negligible or relatively small On the other hand functions of ventilatory capacity and lung volumes (Table IX) exhibit a strong relationship in many cases Such a relationship is found between VC on the one hand and $FEV_{1\,0}$ MVV_{40} MVV_F and MEF on the other The residual quotient (RV/TLC) also shows a number of strong relationships with different functions constituting measures of ventilatory capacity

DISCUSSION

The results presented provide information on the physical work capacity and on lung volumes and also on relations between them in a series of subjects representative of the urban population of Uppsala within the approximate age range of

Table V *Mean value standard deviation and standard error of the mean for the various ventilatory capacity tests after grouping with regard to sex and age*

	$FEV_{1\,0}$ 1			FEV			MVV_F 1			MVV 1			MEF 1		
Age group	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE	M	SD	SE
Males															
57–61 II	3 10	0 68	0 16	71 6	6 4	1 5	140 2	20 0	4 7	104 9	27 4	6 5	517 2	79 7	18 8
62–66 III	2 80	0 56	0 13	67 4	10 5	2 5	116 6	30 9	7 3	92 4	23 4	5 5	500 8	84 9	20 0
67–71 IV	2 80	0 41	0 13	68 8	8 4	2 6	106 4	14 5	4 6	85 0	18 1	5 7	482 0	76 3	24 1
Females															
57–61 II	2 30	0 31	0 07	76 1	6 5	1 4	89 7	17 4	3 8	72 0	12 1	2 7	427 9	47 7	10 4
62–66 III	2 00	0 33	0 07	70 8	8 0	1 8	82 7	17 2	3 9	66 3	11 6	2 6	371 8	79 8	17 9
67–71 IV	2 10	0 38	0 12	73 6	5 [illegible]	1 8	85 6	17 5	5 5	69 0	14 9	4 7	386 5	66 5	21 0

Table VI *Regression equations and residual standard deviations (R S D) for the determinations of lung volume*

The significance level of the regression equations is denoted by asterisks 0 01 < p ≤ 0 05 0 001 < p ≤ 0 01
Insignificant regression coefficients have been placed within brackets

	Sex	Regression coefficients				
		Age y	*Height cm*	*Weight kg*	*Constant*	R.S D
TLC	♂	(+0 031±0 030)	+0 081±0 0 0	(−0 000±0 013)	− 9 834	0 82
	♀	(+0 013±0 0 0)	+0 067±0 012	−0 021±0 008	− 5 96	0 50
FRC	♂	+0 049±0 0?1	+0 081±0 014	−0 038±0 010	−11 170	0 59
	♀	+0 033±0 015	−0 045±0 009	−0 0 3±0 000	− 5 656	0 39
RV	♂	+0 042±0 015	+0 044±0 010	−0 014±0 007	− 7 202	0 41
	♀	+0 035±0 011	+0 030±0 007	−0 009±0 005	− 5 0?1	0 29
FRC/TLC	♂	+0 506±0 195	+0 658±0 131	−0 037±0 088	−47 874	5 4
	♀	+0 600±0 236	+0 295±0 140	−0 ?94±0 095	−18 012	6 1
RV/TLC	♂	+0 494±0 174	+0 243±0 117	−0 222±0 079	−?2 278	4 8
	♀	+0 727±0 186	(+0 155±0 110)	(−0 013±0 075)	−33 517	4 8
VC	♂	(−0 032±0 070)	+0 046±0 013	+0 0?1±0 010	−3 515	0 62
	♀	−0 029±0 013	+0 034±0 008	(−0 003±0 005)	−0 757	0 38

55–70 years This series of subjects differs from series upon which similar studies have been carried out previously mainly in being representative of the basic population—including the degree of previous and present physical training—but also in that it comprises ages which have often not been included in previous investigations

The present study gives results which show that W_m decreases with increasing age and which also indicate that $W_{1\,0}$ and probably also W_{130} and $W_{1\,0}$ follow the same course It is clearly evident from previous investigations (1 5 10 13) that the maximal achievement capacity for circulation limited work (which in our study is most closely represented by W_m) decreases with increasing age and that this also holds for the maximal heart rate (HR_m) It has generally been found however that submaximal indirect measures of physical work capacity e g W_{170} or $W_{1\,0}$ are relatively similar in different age groups indicating that the reduction of $W_{m\,x}$ with increasing age depends on the reduction of HR_m In general however previous studies have not been based on material representative of the population and in hardly any case has there been strict representation

Holmgren (8) among others has shown in *young physically well trained individuals that there* is a strong relationship between respiratory and circulatory function variables No such relationship was found in our series This absence of a strong relationship did not appear to have been due to any uncertainty in the methods an analysis of which showed that the reproducibility of our determinations was good This applied both to determinations made on one and the same occasion

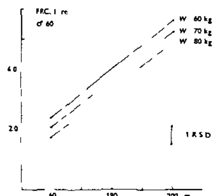


Fig 1 Nomogram illustrating the influence of weight on FRC in men One R.S D is plotted in the figure

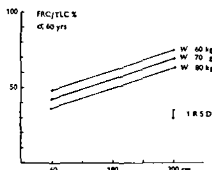


Fig Nomogram illustrating the influence of weight on FRC TLC in men One R S D is plotted in the figure

Table VII *Correlation coefficients with significance asterisks (*) between indices of physical work capacity and lung volumes*

0 01 < p ≤ 0 05

	Sex	VC	RV	FRC	TLC	RV/TLC	FRC/TLC
W_{130}	♂	0 437	−0 007	0 134	0 239	−0 ?07	−0 019
	♀	0 187	−0 034	−0 064	0 07	−0 139	0 032
W_{150}	♂	0 398	−0 055	0 065	0 198	−0 225	−0 084
	♀	0 222	−0 105	−0 012	0 074	−0 264	−0 12
$W_{1\ 0}$	♂	0 433	−0 146	−0 1	0 21	−0 365	−0 34
	♀	0 380	0 086	0 127	0 202	−0 061	0 014
$W_{m\ x}$	♂	0 205	0 009	0 008	−0 023	0 06	0 037
	♀	0 402	−0 016	0 061	0 261	−0 328	−0 199

Table VIII *Correlation coefficients with significance asterisks (*) between indices of physical work capacity and respiratory function*

0 01 < p ≤ 0 05

	Sex	$FEV_{1\ 0}$	FEV	MVV_F	MVV_{40}	MEF
W_{130}	♂	0 256	−0 114	0 169	0 198	0 062
	♀	0 263	0 177	0 094	0 122	0 015
W_{150}	♂	0 273	0 035	0 161	0 21	0 059
	♀	0 31	0 167	0 129	0 098	0 119
$W_{1\ 0}$	♂	0 525	0 320	0 387	0 482	0 171
	♀	0 2	0 124	−0 036	−0 126	0 015
W_{ma}	♂	0 279	0 174	0 212	0 348	0 154
	♀	0 351	0 006	0 098	0 111	0 058

and to those made on separate occasions (4) The weak relationship between respiratory and circulatory function variables in our series may instead be due to the fact that the physiological functional regression with increasing age may have different time courses for the respiratory and the circulatory systems

We have analysed this problem by calculating individual changes in circulatory measures (W_{130} etc) and ventilatory measures (MVV etc) occurring during the period 1961–1966 (3) The reductions were found to be somewhat greater for the circulatory (approximately 10–25) than for the ventilatory measures (approximately 6–9 %)

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Table IX *Correlation coefficients with significance asterisks (*) between indices of lung volumes and respiratory function*

0 01 < p ≤ 0 05 0 001 < p ≤ 0 01 0 001 > p

	Sex	$FEV_{1\ 0}$	FEV	MVV_F	MVV	MEF
VC	♂	0 763	−0 089	0 677	0 598 *	0 576
	♀	0 783	0 03	0 591 *	0 61	0 541
RV	♂	−0 108	−0 513	−0 155	−0 1 9	−0 168
	♀	0 024	−0 31	−0 004	−0 011	−0 094
FRC	♂	0 021	−0 626	−0 0?6	0 014	−0 061
	♀	0 180	−0 275	0 132	0 133	0 001
TLC	♂	0 415	−0 38	0 338	0 28	0 236
	♀	0 52 *	−0 144	0 388	0 387	0 32
RV/TLC	♂	−0 544	−0 367	−0 567	−0 465	−0 494*
	♀	−0 437	−0 312	−0 141	−0 141	−0 301
FRC/TLC	♂	−0 342	−0 587 *	−0 346	−0 249	−0 307
	♀	−0 187	−0 287	−0 361	−0 365	−0 432

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Table VII *Correlation coefficients with significance asterisks (*) between indices of physical work capacity and lung volumes*

0 01 < p ≤ 0 05

	Sex	VC	RV	FRC	TLC	RV/TLC %	FRC/TLC %
W_{130}	♂	0 437	−0 007	0 134	0 239	−0 207	−0 019
	♀	0 187	−0 034	−0 064	0 07	−0 139	0 032
W_{150}	♂	0 398	−0 055	0 065	0 198	−0 2'5	−0 084
	♀	0 222	−0 105	−0 012	0 074	−0 264	−0 12
W_{170}	♂	0 433	−0 146	−0 1	0 21	−0 365	−0 34
	♀	0 380	*0 086*	0 1'7	*0 202*	*−0 061*	*0 014*
W_{ma}	♂	0 205	0 009	0 008	−0 023	0 06	0 037
	♀	0 402	−0 016	0 061	0 261	−0 328	−0 199

Table VIII *Correlation coefficients with significance asterisks (*) between indices of physical work capacity and respiratory function*

0 01 < p ≤ 0 05

	Sex	$FEV_{1\,0}$	FEV	MVV_F	MVV_{40}	MEF
W_{130}	♂	0 256	−0 114	0 169	0 198	0 062
	♀	0 263	0 177	0 094	0 122	0 015
$W_{1\,0}$	♂	0 273	0 035	0 161	0 21	0 059
	♀	0 31	0 167	0 129	0 098	0 119
$W_{1\,0}$	o	0 525	0 320	0 387	0 482	0 171
	♀	0 2	0 1 4	−0 036	−0 126	0 015
W_m	♂	0 279	0 174	0 212	0 348	0 154
	♀	0 351	0 006	0 098	0 111	0 058

and to those made on separate occasions (4) The weak relationship between respiratory and circulatory function variables in our series may instead be due to the fact that the physiological functional regression with increasing age may have different time courses for the respiratory and the circulatory systems

We have analysed this problem by calculating individual changes in circulatory measures (W_{130} etc) and ventilatory measures (MVV etc) occurring during the period 1961–1966 (3) The reductions were found to be somewhat greater for the circulatory (approximately 10–25 %) than for the ventilatory measures (approximately 6–9 %)

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4 — Unpublished observations

Table IX *Correlation coefficients with significance asterisks (*) between indices of lung volumes and respiratory function*

0 01 < p ≤ 0 05   0 001 < p ≤ 0 01   0 001 > p

	Sex	$FEV_{1\,0}$	FEV	MVV_F	MVV_{40}	MEF
VC	♂	0 763	−0 089	0 677	0 598	0 576
	♀	0 783	0 03	0 591 *	0 61	0 541
RV	o	−0 108	−0 513	−0 155	−0 129	−0 168
	♀	0 024	−0 31*	−0 004	−0 011	−0 094
FRC	o	0 021	−0 6'(	−0 026	0 014	−0 061
	♀	0 180	−0 275	0 132	0 133	0 001
TLC	♂	0 415	−0 38	0 338	0 28	0 36
	♀	0 52	−0 144	0 388	0 387	0 3'
RV/TLC	♂	−0 544	−0 367	−0 567 *	−0 465	−0 494
	♀	−0 437	−0 312	−0 141	−0 141	−0 301
FRC/TLC /	♂	−0 342	−0 587	−0 346	−0 249	−0 307
	♀	−0 187	−0 287	−0 361	−0 365	−0 432

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EFFECT OF FIVE YEARS AGEING ON VENTILATORY CAPACITY AND PHYSICAL WORK CAPACITY IN ELDERLY PEOPLE

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Abstract The effect of five years ageing on the ventilatory capacity and physical work capacity is studied on a sample of apparently healthy individuals of ages 57-71 years, representative of the population The physiological functional regression for maximal expiratory volume per 1 0 second and maximal voluntary ventilatory capacity was about 6-9% for both sexes and for physical work capacity somewhat greater viz. 9-13% for the men and about 10-25% for the women This finding is assumed to be due to the fact that the regression in physiological function takes place more rapidly for the circulatory than for the respiratory system. In this series a correlation which has not been reported previously was found between work load at a given pulse rate and age

The aim of the investigation presented below was to study in elderly apparently healthy men and women the effect of five years ageing on the ventilatory capacity and physical work capacity The subjects of this study—who constituted a representative sample of the population of Uppsala urban district within the age range in question—were included in the series which formed the basis of previous studies on normal values of ventilatory capacity lung volumes and physical work capacity (3 4)

The composition of the series with regard to sex age and anthropometric data was identical with that of the series of the previous study concerning physical work capacity and lung volumes The five year follow up investigation with regard to ventilatory capacity was based on 97 subjects (46 men and 51 women) and with regard to physical work capacity 84 subjects (42 of each sex)

For a description of the method reference may be made to the two previous papers in this series mentioned above

RESULTS

It is evident in Table I that the difference between the mean values of vital capacity in men of ages 57–71 years for the years 1961 and 1966 was 0 12 1 or 2 9% of the mean vital capacity value obtained in 1961 The table also shows the difference for the three age groups separately This difference appeared to show no relationship with age within the age range in question

For $FEV_{1\ 0}$ the difference was 0 28 l or 9 2 % for MVV_{40} 8 56 l or 8 9% and for MEF 3 91 l or 0 8

Table II gives the corresponding data for the women For VC the difference was on the average 0 09 l or 3 1% for $FEV_{1\ 0}$ 0 20 l or 8 7% for MVV_{40} 4 39 l or 6 1% and for MEF 11 28 l or 2 8 %

Thus for both sexes it was found that the difference was relatively smaller for VC and MEF than for $FEV_{1\ 0}$ and MVV_{40}.

Figs 1–4 are complementary to Tables I and II The development in the individual subjects with regard to VC $FEV_{1\ 0}$, MVV_{40} and MEF during the five year period is illustrated The physiological functional regression is numerically greater for the $FEV_{1\ 0}$ and MVV_{40} than for VC of MEF tests

Figs 5–9 show with the subjects divided into sex and age groups both the absolute mean value for the group for the respective lung function tests for the years 1961 and 1966 and the difference in per cent of this mean value from the calculated "normal mean value" obtained from comparative series In the case of VC $FEV_{1\ 0}$, MVV_{40} and MVV_{F} these comparative series were

Table I *Difference in litres between values for years 1961 and 1966 for the men*

The figures in parentheses represent the mean differences in per cent of the 1961 mean values for the respective lung function variables

Age groups	VC			FEV1 0			MVV40			MEF		
	M	S D	S E	M	S D	S E	M	S D	S E	M	S D	S E
57–61	0 23 (5 1)	0 30	0 07	0 33 (9 7)	0 23	0 05	5 39 (5 0)	5 51	1 30	−4 12 (8 0)	30 98	7 51
62–66	0 00 (0 0)	0 37	0 08	0 25 (8 3)	0 35	0 08	10 17 (10 2)	13 04	3 08	18 06 (3 5)	47 53	11 20
67–71	0 12 (3 0)	0 17	0 05	0 25 (8 6)	0 25	0 08	11 67 (12 4 ⁄)	12 37	4 12	−6 82 (14 8)	38 62	11 67
57–71	0 12 (2 9 ⁄)	0 32	0 04	0 28 (9 2)	0 28	0 04	8 56 (8 9)	16 43	2 45	3 91 (0 8)	40 72	6 00

Table II *Difference in litres between values for years 1961 and 1966 for the women*

The figures in parentheses represent the mean differences in per cent of the 1961 mean values for the respective lung function variables

Age groups	VC			FEV1 0			MVV40			MEF		
	M	S D	S E	M	S D	S E	M	S D	S E	M	S D	S E
57–61	0 07 (2 3)	0 24	0 05	0 16 (6 6)	0 25	0 05	3 71 (4 9)	7 81	1 70	4 52 (1 0)	35 94	7 84
62–66	0 10 (3 4)	0 29	0 06	0 21 (9 5)	0 31	0 07	5 05 (7 3)	10 51	2 35	16 25 (4 1)	44 12	9 86
67–71	0 1? (4 2)	0 23	0 07	0 25 (11 3)	0 20	0 06	4 70 (6 7)	11 45	3 62	15 50 (4 0)	31 13	9 84
57–71	0 09 (3 1)	0 27	0 03	0 20 (8 7)	0 26	0 03	4 39 (6 1)	9 52	1 33	11 28 (2 8)	38 26	5 35

those published from Goteborg (1) and in the case of MEF those published by Wright and McKerrow (6) and by Lockhardt et al (5) i e the control series which have been used hitherto at the University Hospital Uppsala

For the function parameters which were compared with the Goteborg series our groups exhibited practically throughout a mean value which was below that calculated from the control material while for the parameter MEF which was

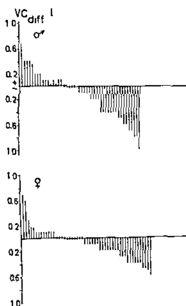


Fig 1 Difference for VC between determinations for years 1961 and 1966 (diff 1966–1961) for each of the men and women included in the series

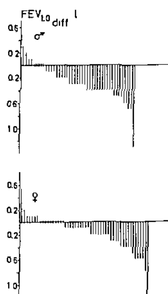


Fig 2 Difference for FEV between determinations for years 1961 and 1966 (diff 1966–1961) for each of the men and women included in the series

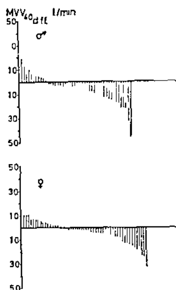


Fig 3 Difference for MVV_{40} between determinations for years 1961 and 1966 (diff 1966–1961) for each of the men and women included in the series

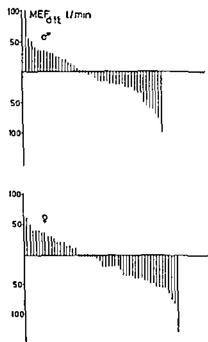


Fig 4 Difference for MEF between determinations for years 1961 and 1966 (diff 1966–1961) for each of the men and women included in the series

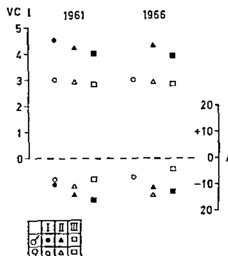


Fig 5 Mean values for VC for groups classified according to age and sex, obtained in the years 1961 and 1966 The left ordinate gives absolute values. The right ordinate gives the difference in per cent from normal mean values obtained from a comparative series (see text)

compared with the British series the opposite was found

Tables III and IV give—with the series divided into sex and age groups—mean values for W_{130} W_{150} $W_{1\,0}$ and W_m both for the year 1961 and for 1966 and also the differences which occurred

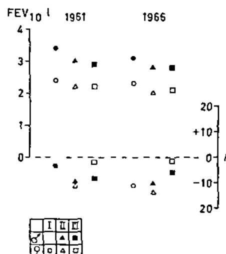


Fig 6 Mean values for FEV for groups classified according to age and sex obtained in the years 1961 and 1966 The left ordinate gives absolute values. The right ordinate gives the difference in per cent from normal mean values obtained from a comparative series (see text)

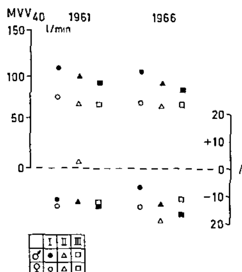


Fig 7 Mean values for MVV_{40} for groups classified according to age and sex obtained in the years 1961 and 1966 The left ordinate gives absolute values The right ordinate gives the difference in per cent from normal mean values obtained from a comparative series (see text)

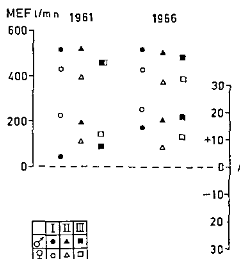


Fig 9 Mean values for MEF for groups classified according to age and sex obtained in the years 1961 and 1966 The left ordinate gives absolute values The right ordinate gives the difference in per cent from normal mean values obtained from a comparative series (see text)

for these function parameters during the five year period The tables also give the number of individuals whose work loads at the respective pulse rates were lower and higher respectively in the year 1966 than in 1961 It is evident from these

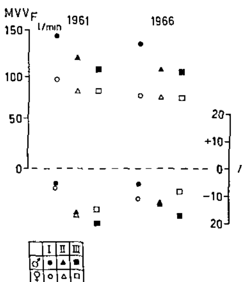


Fig 8 Mean values for MVV_F for groups classified according to age and sex obtained in the years 1961 and 1966 The left ordinate gives absolute values The right ordinate gives the difference in per cent from normal mean values obtained from a comparative series (see text)

tables that all groups showed a negative difference i e the work load of the group at the given pulse rate decreased during the five year period When the series was treated regardless of age grouping the difference for the men was 12 9% for W_{130} 10 8% for W_{150} 9 1% for W_{170} and 11 8% for W_m The corresponding values for the women were 24 0% 19 1% 7 5% and 18 8% respectively These differences were significant for both sexes The differences were also often significant when the series was divided into age groups in spite of the fact that the number of individuals in the groups was often relatively small

Figs 10–12 are complementary to Tables III and IV The development in the individual subjects with regard to W_{130} W_{150} and W_{170} during the five year period is illustrated The figures show that the physiological functional regression for the respective function parameters was relatively large and on statistical analysis the tendencies were found to be significant

Double determinations of physical work capacity were performed on altogether 21 randomly selected subjects representing all three age groups The work tests were carried out by the same method on two consecutive days The mean values for both men and women were higher on the

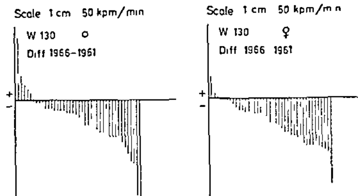


Fig 10 Difference for W_{130} between determinations for years 1961 and 1966 (diff 1966–1961) for each of the men and women included in the series.

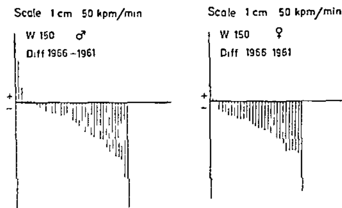


Fig 11 Difference for W_{150} between determinations for years 1961 and 1966 (diff 1966–1961) for each of the men and women included in the series.

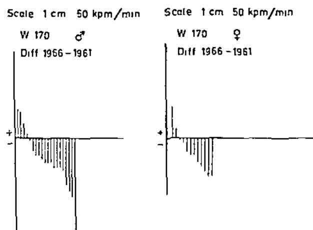


Fig 12 Difference for W_{170} between determinations for years 1961 and 1966 (diff 1966–1961) for each of the men and women included in the series

second day The results are shown in Table V These higher values on the second day might reasonably be explained by the training effect of the work test on the first day in persons with a low degree of physical training

DISCUSSION

Much information has been published previously with regard to the relationship between static and dynamic factors of the oxygen transport capacity and to the importance of its circulatory and res

Table III *Mean values kpm/min for W_{130} W_{150} W_{170} and W_{max} for the men for the years 1961 and 1966 and also the difference between the values at these two dates in per cent of the 1961 values*

Age group I 57–61 years II 62–66 years III 67–71 years
Statistical significance is indicated by asterisks as follows * 0 01 < p ≤ 0 05 0 001 < p ≤ 0 01 *0 001 ≥ p

	Age group	Kpm/min 1961	Mean value 1966	Difference kpm/min 1966–1961			Diff of 1961 value	No of individuals with		
				Mean	S D	S E		negative diff	unchanged value	positive diff
W_{130}	I	720 6	613 9	− 106 7	124 7	29 4	14 8	16	0	2
	II	620 0	565 8	− 54 2	121 1	35 0	8 7	10	0	2
	III	646 4	557 3	− 89 1 *	100 2	30 2	13 8	9	1	1
	I+II+III	671 2	584 6	− 86 6	118 2	18 5	12 9	35	1	5
W_{150}	I	912 2	800 6	− 111 6	122 6	28 9	12 7	16	0	2
	II	790 0	732 7	− 57 3	108 8	32 8	7 3	8	0	3
	III	860 0	758 1	− 101 9 **	80 1	28 3	11 9	8	0	0
	I+II+III	864 6	771 1	− 93 5	109 0	17 9	10 8	32	0	5
W_{170}	I	1098 3	990 4	− 107 9	131 6	38 0	9 8	10	0	2
	II	890 0	844 0	− 46 0	106 2	47 4	5 2	3	0	2
	III	973 3	885 0	− 88 3	93 8	54 2	9 1	3	0	0
	I+II+III	1027 5	938 0	− 89 5	115 5	25 8	8 7	16	0	4
W_{max}	I	1033 3	883 3	− 150 0	185 5	43 7	14 5	8	10	0
	II	800 0	733 3	− 66 7	153 7	77 4	8 3	3	8	1
	III	763 6	690 9	− 72 7	127 2	38 3	9 5	3	8	0
	I+II+III	892 7	787 8	− 104 9	160 1	25 0	11 8	14	[illegible]6	1

piratory components The new features of the present study however are that it comprises a longitudinal investigation over a period of five years and that the series is representative of the population To obtain information on the situation in the population concerning both the effect of age on the studied parameters and the trend of this effect—which are dependent on the prevailing living

Table IV *Mean values in kpm/min for W_{130} W_{150} W_{170} and W_{max} for the women for the years 1961 and 1966 and also the difference between the values at these two dates in per cent of the 1961 values*

Age group I 57–61 years II 62–66 years III 67–71 years
Statistical significance is indicated by asterisks as follows 0 01 < p ≤ 0 05 * 0 001 < p ≤ 0 01 * 0 001 ≥ p

	Age group	Kpm/min 1961	Mean value 1966	Difference kpm/min 1966–1961			Diff of 1961 value	No of individuals with		
				Mean	S D	S E		negative diff	unchanged value	positive diff
W_{130}	I	450 0	347 6	− 102 4	77 3	18 7	22 8	14	2	1
	II	399 4	317 6	− 81 8	103 6	25 2	20 5	14	1	2
	III	388 3	[illegible]40 0	− 148 3	36 0	14 7	38 2	6	0	0
	I+II+III	419 3	318 8	− 100 5	85 7	13 6	24 0	34	3	3
W_{150}	I	584 0	489 3	− 94 7	52 4	14 0	16 2	14	0	1
	II	473 3	356 7	− 116 6	113 5	37 8	24 6	8	1	0
	III	516 7	360 0	− 156 7	53 9	2[illegible] 0	30 3	6	0	0
	I+II+III	524 0	423 7	− 100 3	76 4	13 9	19 1	28	1	1
W_{170}	I	671 7	631 7	− 40 0	91 2	37 2	6 0	5	0	1
	II	528 0	460 0	− 68 0	83 2	37 1	12 9	4	0	1
	III	560 0	400 0	− 160 0	—	—	28 6	1	0	0
	I+II+III	60[illegible] 5	557 5	− 45 0	86 3	4 9	7 5	10	0	2
W_{max}	I	577 8	497 2	− 80 6	36 4	8 6	14 0	8	10	0
	II	511 1	400 0	− 111 1	149 1	35 2	21 7	10	8	0
	III	500 0	366 7	− 133 3	163 3	66 6	26 6	4	2	0
	I+II+III	538 1	436 9	− 101 2	99 1	15 3	18 8	22	20	0

Table V *Mean value standard deviation and standard error of the mean for the difference between the two determinations of physical work capacity*

indicates a statistical significance of $0.01 < p \leq 0.05$

	Difference between determination 2-1			Difference in of determination 1	No of individuals with		
	M	S D	S E	M	*positive* diff	*unchanged* value	*negative* diff
Men							
W_{130}	+39 0	66 9	20 2	+7 9	8	0	3
W_{150}	+40 6	51 5	17 1	+5 3.	7	0	2
W_{170}	+42.9	43 6	16 5	+4 43	6	0	1
W_{max}	±0			±0	0	11	0
Women							
W_{130}	+.29	30 2	9 5	+8 4	7	1	2
W_{150}	+5 0	25 3	8 4	+1 37	6	0	3
W_{170}	+6 0	26 1	9 9	+1 29	5	0	2
W_m	±0			±0	0	10	0

conditions—such studies must be carried out on material representative of the population

The results of this study appear to support the tendency found in our previous study that W_m and also W_{130} W_{150} and W_{170} decrease with increasing age In a survey of the literature we have found no evidence that relationships between the work loads at the given pulse rates and age have been considered previously The physiological functional regression appears to take place rather more quickly for the circulatory than for the respiratory system this tendency seems to be slightly more marked for women than for men One reasonable explanation may be that at these ages men maintain their physical activity to a greater extent than women

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JAUNDICE FOLLOWING SPLENECTOMY IN HODGKIN S DISEASE

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From the Cancer Research Institute Aarhus and the Radium Centre Aarhus Denmark

Abstract Splenectomy has been performed in five pa tients suffering from Hodgkin s disease Four of the pa tients developed jaundice after the operation and died in what was considered hepatic coma. Autopsy was per formed in three of the cases. The liver showed fatty degeneration in one case and necrotic changes in two others without signs of lymphogranulomatous infiltrations The pathogenesis is d scussed

The spleen is often enlarged in patients suffering from Hodgkin s disease even in the early phases If the spleen is only slightly enlarged it may not be palpable but the splenomegaly may be dem onstrated by X ray examination In 50 con secutive cases of Hodgkin s disease treated at the Radium Centre in Aarhus splenomegaly was found in 21 patients

It is a well known fact that a single injection of a therapeutic active dose of Vincoblastine (0 15 mg/kg body weight) is often immediately followed by a sharp decline in the number of leukocytes in the peripheral blood (1) The WBC may fall to below 1000 per μl blood and normal values will not be reached until about a week later The bone marrow will however be cellular even in the most leucopenic phase with a hyperplastic granulopoiesis with a shift to the left The mechanism of the leucopenia has not been fully elucidated in spite of a number of experimental investigations (1 2 3) In the abdominal form of Hodgkin s disease" splenomegaly granulocytopenia and a hypercellular marrow are the rule and in such cases the white blood picture will often reach normal values after splenectomy which probably means that a splenogenic bone marrow has existed The purely speculative hypothesis was made that the Vincoblastine produced leucopenia might be due to a splenogenic marrow inhibition intensified by Vincoblastine as the patients resistance to in fections might be lowered subsequent to the leu copenia On this basis splenectomy was performed in four Vincoblastine treated patients suffering from Hodgkin s disease Furthermore it might be possible to treat the patients with larger doses of Vincoblastine after the operation The sple nectomy was in some cases performed in the phase when the leucopenia was most pronounced

The weights of the spleens were 205 g (B F) 288 g (P H G) 320 g (J R J) and 760 g (B L P) Histological examination showed lym phogranulomatous infiltrations in all spleens

The splenectomy was followed by an increase in the leukocyte count but Vincoblastine injec tions induced leucopenia as before the operation One of the splenectomized patients (P H G) is still alive but the other three developed severe jaundice after the operation and died in what was considered hepatic coma At autopsy degenerative changes were found in the liver parenchyma but neither macroscopic nor microscopic lymphogran ulomatous foci nor infiltrations

Splenectomy was performed in a fifth patient case 4 (J R H) suffering from Hodgkin s disease with splenomegaly leucocytopenia and thrombocytopenia combined with a hyperplastic granulopoiesis with a shift to the left in the bone marrow This patient had not received Vincoblastine but he also died jaundiced in hepatic coma shortly after the operation

CASE REPORTS

Case 1 (B F)

A 31 year-old man was admitted to the cancer clinic in Aarhus in July 196? after two years with enlarged lymph nodes on his neck. Biopsy classical Hodgkin s

disease One year later periods of fever accompanied by sweat weakness and loss of weight When the patient was first admitted the disease was found to be localized to the neck He was treated with TEM totalling 20 mg during a period of three weeks with good effect which however was of rather short duration

In 1962 the patient suffered from fever night sweat and loss of weight He was treated ambulatorily with Arthrizine® 600–400–200 mg At this time hepato splenomegaly with leucopenia and enlarged lymph nodes in the hili appeared The patient was treated with steroids (Delcortin starting with 200 mg and falling to 10 mg daily) and X rays In November 1962 progressive anaemia The leucopenia persisted in spite of a cellular granulopoiesis with a shift to the left in the bone marrow As the existence of a splenogenic marrow inhibition was suspected splenectomy was performed on the 4th De cember 1962 Before the operation the patient received two portions of packed blood—during and immediately after the operation 500 ml blood Microscopy of the spleen (205 g) showed classical Hodgkin s disease

After the splenectomy the WBC values became normal for a short period and the patient did better He suf fered constantly from fever however and as he de teriorated in January 1963 he was again hospitalized Treatment with Vincoblastine was started in February 1963 and the patient received 114 mg up to May 1963 without pronounced effect

The patient was discharged but readmitted on June 6 1963 At that time he was pronouncedly jaundiced Serum bilirubin 17 9 GOT 107 GPT 62 and bile pig ment and urobilin were found in the urine

The patient died on June 17 1963 with severe jaun dice Autopsy and histological examination of the organs revealed no sign of Hodgkin s disease The liver was enlarged but without granulomatous infiltrations Micro scopy showed severe centrilobular necroses No fatty metamorphosis and no cholestasis (Prof Steen Olsen)

Case 2 (B L P)

A 20 year-old man observed enlarged lymph nodes on his neck in July 1960 Biopsy classical Hodgkin s disease Enlarged lymph nodes in the abdominal cavity and me diastinum The patient was treated with TEM 25 mg during 14 days with good effect on the enlarged lymph nodes and the general condition

In December 1960 he was readmitted with progressive enlargement of the lymph nodes on the neck weakness and fever The patient was treated intravenously with cyclophosphamide in doses of 300–500 mg daily from December 5 to January 9 totalling 3800 mg with good effect on the lymph nodes and the general condition In September 1961 cough and pains between the scapulas developed X ray examination showed mediastinal tumour He was treated with TEM 20 mg and X rays with good effect on the mediastinal tumour The symptoms returned later in the year Now there was also a general enlarge ment of the superficial lymph nodes and an enlarged spleen The blood picture showed granulocytopenia but the bone marrow was hypercellular with a granulopoiesis with a shift to the left The patient received several blood transfusions Splenectomy was performed on January 24 1963 Microscopy of the spleen (760 g) classical Hodg kin s disease After the operation somewhat higher WBC count Vincoblastine was given from February 9 to May 25 1963 totalling 265 mg

The lymph nodes decreased in size and the patient did comparatively well until June 1963 when he became tired, feverish and slightly jaundiced The serum bilirubin which had hitherto been normal was now 6 23–11 60 GOT 19 GPT 5 and alkaline phosphatases 18 4 KAE. In September 1963 bile pigment and urobilin appeared in the urine

The symptoms progressed and the patient died on September 21 1963 with intense jaundice At autopsy the liver was found to be enlarged with a few very small infiltrates Microscopy showed severe centrilobular necroses with granulocyte infiltrations around the necro tic foci No cholestasis (Prof Steen Olsen)

Case 3 (I R J)

A 21 year-old man was admitted to the cancer clinic in Aarhus for Hodgkin s disease in 196 Since December 1961 enlarged lymph nodes on his neck Biopsy classical Hodgkin s disease When referred to the cancer clinic there was a general enlargement of the superficial lymph nodes and probably also of the lymph nodes in the abdomen The spleen was slightly enlarged The pa tient was treated with TEM 25 mg during 14 days with some effect He was however constantly suffering from pruritus and night sweat and the blood count showed leucopenia He was readmitted to the cancer clinic in October 1962 and treatment with Vincoblastine was started on November 5 1962 From November 5 1962, to May 16 1963 he was given 242 mg Vincoblastine

As the patient now had splenomegaly leucopenia and a hypercellular bone marrow with a shift to the left in the granulopoiesis splenectomy was performed on De cember 4 1962 Microscopy of the spleen (370 g) showed classical Hodgkin s disease After the operation there was some increase in the WBC count The Vincoblastine treat ment was continued but the patient deteriorated and died on July 7 1963 Shortly before he died he developed a severe jaundice The serum bilirubin which had hitherto been normal rose to 3 25–5 0 alkaline phosphatases 19 30 KAE, and urobilin appeared in the urine

At autopsy no sign of Hodgkin s disease was found in the liver which was enlarged with pale parenchyma Microscopy showed slight fatty metamorphosis but no necroses and no cholestasis (Prof Steen Olsen)

Case 4 (J R H)

A 39 year-old man observed an enlarged lymph node on the right side of his neck in 1961 It disappeared but was found again one year later and the patient was ad mitted to the cancer clinic Biopsy classical Hodgkin s disease The patient was treated with X rays to the enlarged lymph nodes on the neck He was doing well for about two years when weakness fever night sweat and pains in the abdomen appeared The spleen was enlarged and there were enlarged lymph nodes in the abdomen There was now anaemia leucopenia and

thrombocytopenia in spite of a hypercellular granulo thrombopoiesis in the bone marrow

Splenectomy was performed on April 18 1963 Microscopy of the spleen (unfortunately not weighed) showed lymphogranulomatous infiltrations The blood values became normal after the splenectomy but progressive jaundice with serum bilirubin as high as 24 5 mg % developed from April 26 Alkaline phosphatases 28 0–27 7 KAE Bile pigment and urobilin appeared in the urine Several transfusions of packed blood were given before and during the operation

The patient died with severe jaundice on May 15 1963 At autopsy the liver was of normal size The cut surface showed a slight degree of congestion Scattered white infiltrates a few millimetres in size were seen Unfortunately no microscopy was performed (Prof Steen Olsen)

DISCUSSION

The remarkable frequency of jaundice in our splenectomized patients (in four out of five) may of course be incidental as jaundice occurs in 4–6 % of patients suffering from Hodgkin s disease (6) However the occurrence of jaundice after splenectomy in four consecutive cases gave rise to some speculations concerning its pathogenesis

All patients had received blood transfusions before and during the operation and a transfusion hepatitis cannot be excluded It is however unlikely as at the same time many of our patients received blood transfusions which did not cause hepatitis in any of them

Three of our patients had been treated with Vincoblastine (Velbe®) but the fourth had not so it is unlikely that Vincoblastine was the cause of the liver disease All patients had received Arthrizine® (Butazolidin®) at some time during their disease but the administration of Arthrizine could not be correlated to the appearance of jaundice Furthermore Arthrizine was given in moderate doses (600 mg the first day 400 mg the second day and 200 mg the third day) (4) No correlation was found between this treatment and the appearance of the jaundice We have not observed jaundice in any of the many patients who were treated with Arthrizine during the same period

If the jaundice was a result of the splenectomy the spleen might have inhibited an agent latently *present in the organism (hepatitis virus?) In that* case the splenectomy could have removed an inhibitor

Another possibility should be considered In 1959 Kaplan and Smithers (5) put forward the theory that Hodgkin s disease was an autoimmune disease resembling runt disease Antibodies against normal tissue including the liver should be produced by the lymphogranulomatous tissue If that were the case it could be assumed that splenectomy would remove an antibody inhibiting factor and that this would be the cause of the liver degeneration seen in our patients This theory is as stated very speculative

It is well known that jaundice in Hodgkin s disease may be caused by several mechanisms It may be a haemolytic jaundice or more frequently the jaundice will be a result of bile stasis either caused by pressure on the biliary ducts of enlarged lymph nodes in porta hepatis or by intrahepatic lymphogranulomatous infiltrations Parenchymatous degeneration is often found in the liver The cause is not known In three of the splenectomized patients mentioned in this paper enlarged lymph nodes were found in porta hepatis but none of the patients developed jaundice before the splenectomy was performed The jaundice in the cases mentioned was hardly conditioned by cholestasis as it appeared at a time when therapy had been started which should induce a decrease of the enlarged lymph nodes

ACKNOWLEDGEMENTS

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As the patient now had splenomegaly leucopenia and a hypercellular bone marrow with a shift to the left in the granulopoiesis splenectomy was performed on December 4 1962 Microscopy of the spleen (320 g) showed classical Hodgkin s disease After the operation there was some increase in the WBC count The Vincoblastine treatment was continued but the patient deteriorated and died on July 7 1963 Shortly before he died he developed a severe jaundice The serum bilirubin which had hitherto been normal, rose to 3 25–5 0 alkaline phosphatases 19 30 KAE, and urobilin appeared in the urine

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ST CHANGES AT EXERCISE IN PATIENTS WITH SHORT P–R INTERVAL

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Abstract Twelve patients with a P–R interval of 0 12 sec or less and no delta wave or ST–T changes in the resting ECG performed a multiple-stage graded exercise test. For each of these patients a control subject of the same age and sex was selected The controls fulfilled the same requirements as the patients except for short P–R interval ST segment amplitudes were not significantly different at rest in the two groups ST-J and ST segment amplitudes in lead CR were however significantly lower in the group with short P–R interval both during and 3 min after exercise This observation is important to keep in mind when evaluating exercise electrocardiograms.

Lown et al (6) have described a syndrome characterized by short P–R interval normal QRS and high incidence of paroxysmal tachycardia In this syndrome there are generally no signs of organic heart disease A shortening of the P–R interval in the absence of the QRS abnormality of pre-excitation also occurs in a wide range of diseases affecting the myocardium e g coronary disease with and without myocardial infarction (2 13) systemic hypertension (11) beri beri (12) hyperthyroidism (12) and anxiety neurosis (9)

Marked ST–T wave changes are frequently seen in the pre-excitation or Wolff–Parkinson–White syndrome and may lead to an erroneous diagnosis of ischemic heart if the delta wave is overlooked (3 5 9) Studies of the ST–T segment in patients with short P–R interval and no myocardial disease are scarce Lown et al (6) did not discuss the prevalence of ST–T changes in their material Reinikainen (8) studied a series of 52 patients with paroxysmal tachycardia and short P–R interval (0 11 sec or shorter) and found no ST–T changes We recently noted that an abnormal ST response during and after exercise not attributable to any of the known causes occasionally was associated with a short P–R interval This prompted us to undertake a systematic study

MATERIAL AND METHODS

A search was made of the complete 1966 ECG file at Serafimerlasarettet for tracings recorded at rest in the supine position and with the following characteristics P–R interval of 0 12 sec or less heart rate below 100 beats/min, positive P wave in leads I and II and no significant delta waves (significant delta wave defined according to (7) as the presence of delta waves in at least two leads and with a duration of 0 03 sec or more in at least one lead) Only the most recent ECG was evaluated in patients with multiple records Sixty three patients 21 men and 42 women age range 18 to 76 and mean age 4_ years fulfilled the criteria. A total of 70 cases were excluded from further analysis in an attempt to eliminate all individuals with conditions known to be associated with ST–T wave changes Five of these patients were excluded because they showed specific ECG abnormalities (three left or right ventricular hypertrophy one bundle branch block one myocardial infarction) Three patients were taking digitalis Finally eight men in ages above 45 and four women above 55 years of age were excluded on grounds of their age alone to minimize the likelihood of including subjects with latent coronary disease Hospital and outpatient clinic charts for all patients included in the study were reviewed.

The file of multiple-stage graded exercise tests was also surveyed Twelve patients three men in ages 19 to 45 and nine women 25 to 51 years old satisfied the criteria listed above with respect both to inclusion and exclusion None had higher blood pressure than 160-95 mm Hg All had normal T waves For each of these patients a control subject of the same age and sex was

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Table I *Heart rate and ST amplitudes at rest and during and after exercise*

			Rest					Exercise				
				CR$_2$		CR$_5$			CH$_3$		CH$_5$	
		Age	Heart rate	J	ST	J	ST	Heart rate	J	ST	J	ST
Short PR	$\bar{X}$	37 1	73 8	15	48	– 02	17	145 3	– 04	28	– 18	– 01
N = 12	SE	3 2	3 2	05	08	02	03	4 1	04	08	03	04
	SD	11 2	10 9	16	26	05	11	14 2	15	27	09	13
Controls	$\bar{Y}$	36 5	79 6	12	38	– 01	15	164 2	00	26	– 09	16
N = 12	SE	3 3	6 1	01	07	01	02	3 9	03	03	02	04
	SD	11 4	21 2	04	23	0.	05	13 6	11	10	08	15
	t	12	84	53	92	51	37	3 31	76	20	2 50	2 97
	p	—	—	—	—	—	—	< 01	—	—	< 05	< 0

		Immediately after exercise					Three min after exercise				
			CR$_3$		CR$_5$			CR$_4$		CR$_5$	
		Heart rate	J	ST	J	ST	Heart rate	J	ST	J	ST
Short PR	$\bar{Y}$	100 5	04	46	– 14	10	88 3	04	39	– 09	03
N = 12	SE	3 0	03	09	01	05	3 1	03	07	01	0?
	SD	10 4	10	30	04	17	10 8	09	24	05	08
Controls	$\bar{X}$	117 9	06	55	– 05	.6	99 3	09	44	– 04	18
N = 12	SE	4 8	02	04	02	06	5 7	01	04	01	05
	SD	16 7	07	13	06	20	19 8	03	15	04	15
	t	3 06	60	93	4 20	2 09	1 69	1 62	62	2 94	3 01
	p	< 02	—	—	< 01	—	—	—	—	< 02	< 02

selected at random from the exercise test files The controls fulfilled the same requirements as the patients except for short P–R interval The P–R interval at rest in the control group ranged from 0 13 to 0 18 sec The mean age was 36 5 years in the control group with a standard error of the mean of 3 7 years Corresponding figures for the group of patients with short P–R interval were 37 1 and 3 2 years

All electrocardiograms were recorded at a paper speed of 50 mm/sec on a four channel high fidelity direct writing recorder with jet galvanometers (Mingograf 42 Elema, Sweden) A 16-lead ECG was recorded at rest (I II III aVR, aVL aVF V and and cor responding CR leads) Four chest leads with the indif ferent electrode placed on the forehead were recorded during bicycle ergometer exerci e (CH$_3$, and) during the 5th min at each load A complete resting ECG was recorded immediately after and 3 min after exercise P–R intervals were measured from the simultaneously recorded leads I II III and CR ST amplitudes were measured to the nearest 0 05 mV (0 5 mm) at the QRS–ST junction (J) and at a point corresponding to the interval end of QRS—end of T The average amplitude in three beats was used in the calculations

RESULTS

ST changes at rest

The sixty three patients corresponded to 1 4% of all patients in the ECG file Thirty three per cent were men All ages (expect pediatric) were represented in the sample and the age distribu tion was similar to that of the total ECG pa tient population Seven of the 63 patients had a history of paroxysmal tachycardia This represents a prevalence of 11% One patient had ECG evidence of a paroxysmal supraventricular tachy cardia

Among the 43 patients who were included in the analysis of ST changes 13 had a maximal ST–J (QRS–ST junction) depression in any lead of 0 5 mm three depression of 1 0 and one of 1 5 mm Two patients in the group of four with ST–J depressions of 1 0 mm or more had horizon tal ST segments They were both women 46 and 51 years old and had P–R intervals of 0 11 and 0 12 sec ST–J depressions were seen predomi nantly in leads II CR$_4$ and CR$_5$ and occasionally in leads I and CR$_7$ The ST segment was upward sloping in all remaining cases

ST changes during and after exercise

Results of the comparison of the ECG response to exercise in the 12 patients with short P–R in terval and in the 12 matched controls are pre

sented in Table I The heart rate at rest was not significantly different but the heart rate during exercise was 19 beats/min higher in the control group ($p < 0.01$) Post-exercise heart rates were also higher in the control group ST segment amplitudes were not significantly different at rest ST segment amplitudes in lead CR were similar in both groups but ST–J and ST segment amplitudes in lead CR were significantly lower in the *group with short P–R interval both during and* after exercise

Three patients two women 34 and 43 years old and one man 45 years old with short P–R interval had horizontal or downward sloping ST segments immediately after exercise These changes persisted 3 min after exercise in one subject The remaining subjects in both groups had upward sloping ST segment In no case was chest pain precipitated by the exercise test

DISCUSSION

The characteristics of the 63 subjects with an ECG at rest only are similar to those of the group *described by Lown et al (6) In both materials* /3 of the patients were women A history of paroxysmal tachycardia was recorded in 10 and 11 % of the cases respectively

The prevalence of horizontal ST depression at rest measuring 1 0 mm or more two out of 43 is statistically not different from zero The same is true of isolated ST–J depressions of 1 0 mm or more The number of patients with minimal ST–J depressions (less than 0 5 mm) was high but observer variability in this range is considerable (1) Thus there was no indication that the rate of significant ST depression at rest is increased in patients with short P–R interval This finding is in agreement with Reinikainen s results (8)

In the 12 subjects with an exercise test the ST–J and ST segment amplitudes in lead CR_5 during and at 3 min after exercise were significantly lower than in the controls All heart rates were higher in the control group This would normally have been associated with larger negative ST–J amplitudes among the controls Analysis of available clinical data did not reveal any significant difference between the two groups with respect to the conditions known to be associated with a short P–R interval as listed in the introduction

It is tempting to speculate to what extent short P–R interval in groups such as those of the present study is related to the pre-excitation syndrome The ST–T changes in pre-excitation are generally considered to be secondary to the conduction abnormality (5) There are also data supporting the view that the short P–R interval *syndrome may be associated with repolarization* abnormalities in the absence of organic heart disease Hilmer (4) has recently described a series of 70 patients with T wave changes and no clinical cardiovascular disease They showed as a group a high incidence of shortened P–R interval and of paroxysmal tachycardia Inverted T waves were in his series frequently normalized during a Valsalva manoeuver Two patients intermittently displayed an unequivocal pre-excitation but ST–T wave changes were present also during normal intraventricular conduction

To sum up this study shows that there may be an over representation of ST changes after exercise in patients with short P–R intervals

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			Rest					Exercise				
				CR₂		CR₅			CH₂		CH₅	
		Age	Heart rate	J	ST	J	ST	Heart rate	J	ST	J	ST
Short PR $N=12$	X	37 1	73 8	15	48	– 02	17	145 3	– 04	?8	– 18	– 01
	S E	3 2	3 2	05	08	02	03	4 1	04	08	03	04
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	t	12	84	53	92	51	37	3 31	76	20	2 50	2 97
	p	—	—	—	—	—	—	< 01	—	—	< 05	< 0?

		Immediately after exercise					Three min after exercise				
			CR		CR₅			CR₂		CR₅	
		Heart rate	J	ST	J	ST	Heart rate	J	ST	J	ST
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	S D	10 4	10	30	04	17	10 8	09	24	05	08
Controls $N=12$	$\bar{X}$	117 9	06	55	– 05	26	99 3	09	44	– 04	18
	S E	4 8	02	04	02	06	5 7	01	04	01	05
	S D	16 7	07	13	06	20	19 8	03	15	04	15
	t	3 06	60	93	4 20	2 09	1 69	1 62	6?	2 94	3 01
	p	< 02	—	—	< 01	—	—	—	—	< 0?	< 02

selected at random from the exercise test files The controls fulfilled the same requirements as the patients except for short P–R interval The P–R interval at rest in the control group ranged from 0 13 to 0 18 sec The mean age was 36 5 years in the control group with a standard error of the mean of 3 7 years Corresponding figures for the group of patients with short P–R interval were 37 1 and 3 2 years

All electrocardiograms were recorded at a paper speed of 50 mm/sec on a four-channel high fidelity direct writing recorder with jet galvanometers (Mingograf 42 Elema Sweden) A 16-lead ECG was recorded at rest (I II III aVR, aVL aVF V and and corresponding CR leads) Four chest leads with the indifferent electrode placed on the forehead were recorded during bicycle ergometer exercise (CH, and) during the 5th min at each load A complete resting ECG was recorded immediately after and 3 min after exercise P–R intervals were measured from the simultaneously recorded leads I II III and CR ST amplitudes were measured to the nearest 0 05 mV (0 5 mm) at the QRS–ST junction (J) and at a point corresponding to the interval end of QRS—end of T The average amplitude in three beats was used in the calculations

RESULTS

ST changes at rest

The sixty three patients corresponded to 1 4% of all patients in the ECG file Thirty three per cent were men All ages (expect pediatric) were represented in the sample and the age distribution was similar to that of the total ECG patient population Seven of the 63 patients had a history of paroxysmal tachycardia This represents a prevalence of 11% One patient had ECG evidence of a paroxysmal supraventricular tachycardia

Among the 43 patients who were included in the analysis of ST changes 13 had a maximal ST–J (QRS–ST junction) depression in any lead of 0 5 mm three depression of 1 0 and one of 1 5 mm Two patients in the group of four with ST–J depressions of 1 0 mm or more had horizontal ST segments They were both women 46 and 51 years old and had P–R intervals of 0 11 and 0 12 sec ST–J depressions were seen predominantly in leads II CR_4 and CR and occasionally in leads I and CR_7 The ST segment was upward sloping in all remaining cases

ST changes during and after exercise

Results of the comparison of the ECG response to exercise in the 12 patients with short P–R interval and in the 12 matched controls are pre

Aca med scand Vol 185 pp 209–210 1969

THE HbO DISSOCIATION IN CARDIOMYOPATHY

E Orinius

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Abst act The oxygen saturation of blood from seven pa tients with cardiomyopathy and seven controls ha been determined after equilibration with 1 1 and 2.1 oxygen gas mixtures No significant differences were found be tween patients and controls nor between smokers and non-smokers

In a preliminary communication Astrup (1) in 1964 described a shift of the oxyhaemoglobin dissociation curve to the left in four patients with cardiomyopathy from one and the same family A shift to the left signifies an impairment of oxygen release and this could possibly be of patho genetic significance in cardiomyopathy because of the high oxygen extraction in the myocardium Therefore it seemed of interest to determine the oxygen dissociation in some patients with car diomyopathy

MATERIAL AND METHODS

The group studied consisted of all patients with cardio-myopathy adm tted to the Department of Medicine Sera f merlasarettet from July 1 1965 to December 31 1966 The diagnosis was based on a history of chroni cardiac symptoms and physical find ngs which after in estigation including heart catheterisation and sometimes also angio cardiography could not be attributed to hypertension congen tal or rheumatic heart disease constrictive peri card tis or any heart affecting general disease including alcoholism In order to reduce the possibility of includ ing patients with coronary heart disease an onset of the disease prior to the age of 40 in men and 50 in women, or a family history of heart d sease or sudden death before these ages in the parents or siblings was also required These criteria were fulfilled by three men aged 32-55 and four women aged 31-50 Three were smokers four were not A control group was selected among hospital staff and patients and the controls were matched with regard to sex age and smoking habits Four were patients with diagnoses of lumbago psycho-neuros s, pulmonary tuberculosis and mild hypertension respectively

The subjects had refrained from smoking f r at lea t 12 hours when 5 ml venous blood wa drawn into a hep-arinized tube After 5 min of mixing 0 1 ml was transferred to each of four tonometers (according to Astrup Radiometer Copenha en) The tonometers had then been perfused by an equilibration gas mixture for 5 min at 38 C Two gas mixtures were used containing 1 1% and 2.1% oxygen respectively 5 8 carbon di oxide and nitrogen to 100% (analyses by Haldane tech nique and gas chromatography) After 30 and 20 min respectively in the tonometers the blood from the four tonometers was transferred to three cuvettes, and the oxygen saturation was determined by the method de scribed by Holmgren and Pernow (4) Fully saturated blood for this determination was obtained by equilibra tion with oxygen in the tonometers for 5 min and the fully desaturated blood had been in the tonometers for the same time before adding sodium dithionite The spectrophotometer was of the Beckman B type and the isobestic point had been determined to 505 millimicrons

Duplicate determinations were performed Thus the oxygen saturat on value for each of the two equilibration gas mixtures was usually the mean of three spectro photometer determinations after two equilibration pro cedures each

In order to determine the random error of the method another tube was simultaneously f lled with blood from six of the patients and controls and then treated in the same way

RESULTS

The results are presented in Fig 1 The mean oxygen saturation value after equilibration with the 1 1 % oxygen mixture was 10 7 ± 0 6 for the cardiomyopathy patients and 10 1 ± 1 1 % for the controls The corresponding values for the 2 1 oxygen mixture were 22 9 ± 0 7 and 22 6 ± 0 7 % Neither of these differences is significant

The mean oxygen saturation value after equi libration with the 1 1 oxygen mixture was 10 7 ± 0 4 % for smokers and 10 2 ± 0 6 % for

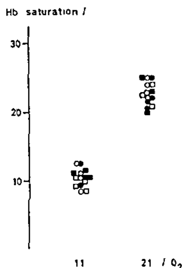


Fig 1 Hb saturation values after equilibration with 1 1 and 2 1 oxygen in patients with cardiomyopathy (○) and controls (□) Filled symbols for smokers open for non smokers

non smokers The corresponding figures for the 2 1 % oxygen mixture were 22 6±0 8% and 23 0±0 6% respectively The differences are not significant

There were no significant differences between samples I and II in the six duplicate determinations 10 2±0 6° against 9 6±0 3% for the 1 1° oxygen mixture and 22 2±0 5% against 21 4±0 4% for the 2 1 mixture The standard error of a single determination was calculated at 1 6 and 1 4% for the 1 1 and 2 1 mixtures respectively

DISCUSSION

In this study no evidence could be found of a shift to the left of the oxygen dissociation curve in a group of seven patients with cardiomyopathy This result is contrary to Astrup s findings Astrup s patients were smokers the blood samples had been taken without any smoking abstinence (3) and the shift to the left of the oxygen dissociation curve could therefore be explained as a carbon monoxide effect (2) This interpretation agrees with the fact that the smokers of the present study after 12 hours of smoking abstinence did not differ from the non smokers in oxygen saturation values

ACKNOWLEDGEMENT

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Acta med scand Vol 185 pp 211–213 1969

NORMAL OR ELEVATED HAEMOGLOBIN VALUES IN CHRONIC RENAL FAILURE

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Abstract Seven patients with chronic renal failure and unexpectedly high haemoglobin values are reported It is concluded that the anaemia producing principle is possibly compensated by a rise in erythropoietin production due to impaired renal blood flow

A report is given below of seven patients with slight to moderate renal insufficiency in whom instead of the expected anaemia normal or slightly elevated haemoglobin values were found

Most patients with chronic renal insufficiency suffer from anaemia This is probably due to suppression of erythropoiesis resulting from the toxic action of a retention product on red cell production or from the lack of erythropoietin In some instances a haemolytic component has been found The anaemia is usually normochromic and normocytic (6 8) The severity of the anaemia correlates fairly well with the degree of renal failure (14)

Polycythaemia has been reported in patients with various renal diseases hypernephroma (2 7 10) polycystic kidney disease (2 5 13) hydronephrosis (7) and renal artery stenosis (4 9) The connection between raised erythropoietin levels and polycythaemia has been established in many cases (12) Christiansen et al (1) reported nine cases with polycythaemia and nephropathia suffering from diabetes mellitus or arterial hypertension It was concluded that reduced renal blood flow on account of arteriosclerotic changes of the intrarenal arteries was an obviously possible cause of stimulation of the production of erythropoietin Hilden and Hilden (3) have recently reported that the haemoglobin value is higher in hypertensive patients than in normotensive patients with comparable degree of renal failure

In most of the reported cases of polycythaemia associated with renal carcinoma or benign renal disease there has been no renal insufficiency

MATERIAL

During the period 1961–1967 1078 patients were admitted to the Renal Ward of the Fourth Medical Department University of Helsingfors All patients with creatinine values higher than 1.5 mg/100 ml and haemoglobin values higher than 15 5 g/100 ml in males and 14 5 in females were further studied Patients with only occasional high values and patients showing signs of polycythaemia due to anoxia or dehydration were excluded None of the patients had been treated with anabolic steroids The material consists of six males and one female The clinical diagnosis and laboratory data are illustrated in Table I

RESULTS

The table shows that all seven patients suffer from slight to moderate renal insufficiency Bone marrow biopsy was made in four cases Increased erythropoiesis was found in two and normal marrow in the other two cases Oxygen saturation was tested in two cases and found normal The serum iron values were normal in all cases Nothing abnormal was found in the leucocyte and thrombocyte values Proteinuria was found in five cases and haematuria in two Total red cell volume was investigated in four cases and found slightly elevated in three and normal in one Erythropoietin determinations have not been made No patient suffered from diabetes mellitus All patients had moderate arterial hypertension and six were treated with antihypertensive drugs

Table I Data for seven patients with chronic renal failure and unexpectedly high haemoglobin values

Pat no	Initials	Sex	Age (y)	Diagnosis	Haemoglobin (g/100 ml)	Erythrocytes (mill/mm³)( )	Haematocrit	Serum creatinine (mg/100 ml)	Creatinine clearance (ml/min)	Serum urea (mg/100 ml)	Phenol sulphthalein excretion (in 2 h)	Blood pressure	Duration of renal failure
1	E L	♂	37	Chronic glomerulonephritis	16 4	5 19	47	2 8	48	45	42 7	175/120	2 years
2	A R	♂	47	Chronic glomerulonephritis	15 6	4 93	48	3 1	21	87	28	160/110	2 years
3	P S	♀	57	Chronic interstitial nephritis	14 8	4 4	43	2 8	25	76	12	160/110	4 years
4	S M	♂	42	Chronic interstitial nephritis	15 9	5 13	47	2 5	35	61	28 5	170/100	Recently discovered
5	J H	♂	35	Arteriosclerotic kidney disease St p nephrectomiam l dx	15 6	5 25	48	1 6	74	55	67 8	150/110	2 years
6	L A	♂	42	Arteriosclerotic kidney disease	16 3	5 10	49	1 65	55	38	55	1(0/1 0	Recently discovered
7	M H	♀	40	Polycystic kidney disease	18 8	5 95	53	2 8	59	56	27 6	190/1°0	1 year

Renal biopsy was performed in cases 1 2 4 and 6 Considerable arteriosclerotic changes in the vessels and glomerular changes were found in all cases In one case a large juxtaglomerular apparatus was seen In another case the afferent arteriole in many glomeruli was surrounded by a multitude of cells indicating a possibly enlarged juxtaglomerular apparatus In the two other cases no signs of enlargement could be seen

DISCUSSION

The connection between production and destruction of red blood cells and renal diseases has long been known It is an established fact that some renal diseases may produce polycythaemia and that patients with chronic renal failure almost invariably suffer from anaemia The erythropoietin is at least partly produced in the kidneys probably in the juxtaglomerular apparatus (11) The secretion of erythropoietin is affected by the renal blood flow and a reduced rate of perfusion may cause an increase in erythropoietin production (12)

Moderate hypertension was found in all our patients This is in conformity with the observation of Hilden and Hilden that higher haemoglobin values are found in hypertensive patients than in normotensive patients with the same degree of renal failure (3) Seven of their 39 hypertensive patients fulfill our criteria of renal failure and higher than expected haemoglobin values Of these seven patients five suffered from essential hypertension and two from chronic pyelonephritis

One of our patients suffers from polycystic kidney disease His laboratory data are reported only to show that the haemoglobin values may remain elevated in spite of progressive renal failure

In the other six patients a reduced renal blood flow is a possible cause of stimulation of erythropoietin It seems reasonable to assume that the anaemia producing factors are in some way compensated by this possible increase in erythropoietin production Why this is so in only a small proportion of all patients with chronic renal failure remains obscure

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PULMONARY REACTION TO NITROFURANTOIN

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Abstract Two cases of pulmonary reaction to nitro furantoin with leucocytosis and eosinophilia are reported Pleural effusion and diffuse pulmonary infiltration were found in both cases Rapid improvement was noted when nitrofurantoin was discontinued The syndrome is characterized as an acute febrile noncardiac pulmonary oedema. The clinical picture and differential diagnoses are discussed The authors explain the syndrome as a hypersensitive type of reaction

Nitrofurantoin is one of the drugs most widely used in the treatment of urinary infections Various side effects have been described hypersensitivity reactions megaloblastic anaemia haemolytic anaemia aplastic anaemia skin rash nausea vom iting headache polyneuritis transient deafness tinnitus enuresis decrease in spermatogenesis and alcohol intolerance (9)

Pulmonary infiltration and pleural effusion due to nitrofurantoin sensitivity were first described by Israel and Diamond in 1962 (6) Since then about 20 similar cases have been reported (1-2 4 7-8 10-15 17) The patients usually tolerated the drug well during the first course of treat ment Later during the same uninterrupted period of treatment or on starting a second cure an anaphylactic reaction to the drug developed Some hours after taking the drug the patients usually experienced sudden dyspnoea malaise and fever The symptoms promptly disappeared after dis continuing the drug Rales but no wheezing often accompanied the dyspnoea Of the 23 cases re ported diffuse pulmonary infiltration in the lower part of one or both lungs was found in ten cases Of these patients one had pleural effusion too Pleural effusion alone was found in two cases and normal chest roentgenogram in four No details of the X rays were reported in seven cases A total leucocyte count of over 10 000 was reported in 12 cases and eosinophilia of more than 5% in 11 while no information about the leucocytes was given in seven cases Skin rash accompanied the other symptoms in four cases The primary diagnoses in the reported cases have usually been pulmonary oedema pneumonia or pulmonary embolism and therapy has been directed against these diseases During the critical period nitro-furantoin has been discontinued and rapid im provement has invariably taken place No fatal outcome has been reported

Since nitrofurantoin is extensively used in the treatment of urinary infections we think it im portant to draw attention to this rare side effect by reporting two cases treated at our hospital in 1967 and 1968

CASE REPORTS

Case 1 A A born 1903

A 64 year-old female office clerk was treated for eczema in 1939 and later dermatitis presumably of allergic aetiology has occasionally appeared. Thirty years ago she had her first attack of cystitis and since then there have been occasional attacks which have sometimes been treated with sulphonamide This drug she has tolerated well. N trofurantoin has not been prescribed

In March 1968 she developed symptoms of urinary infection Bacteria and leucocytes were found in the urine She was first unsuccessfully treated with sulphon amide Then her physician prescribed nitrofurantoin 50 mg three times da ly which she took for one week and the symptoms of cystitis d sappeared. During the cure she did not feel well and perspired profusely After one week of treatment, on April 5 1968 she had fever and malaise There were no symptoms of respiratory infec tion She was visited by another doctor who prescribed tetracycline 250 mg four times daily and the nitro-furantoin was discontinued. Next morning the ymptoms had d sappeared but tetracycl ne was continued until Apr l 7 She then consulted th latter physician who ad ised her to stop the tetracycline med cation and start



Fig 1 a and *b* Case 1 Chest roentgenogram April 7 showing pleural effusion and diffuse especially basal infiltrations of the parenchyma Nitrofurantoin 100 mg taken the same day

on nitrofurantoin again About 5 p m on the same day she took 50 mg of nitrofurantoin and the same dose about 9 30 p m Within an hour she developed severe dyspnoea but only mild cough and no sputum She was visited by a doctor who gave her an injection of glyphylline without effect and later that night she was sent to the emergency department of the University Central Hospital of Helsingfors On admission she was severely pnoeic and the respiratory sounds were depressed temperature was 38 C The blood pressure was 170/90 and the pulse rate 82 min The ECG showed signs of combined left and right ventricular strain Nitrofurantoin was discontinued and she was treated with digitalis, furosemid morphine and oxygen, and in a few hours she was virtually symptom free The chest roentgenogram (Fig 1 *a* and *b*) showed diffuse infiltration in both lungs and effusion in the right pleural cavity The ESR was 32 mm in one hour the haemoglobin 14 1 g/100 ml. The white cell count was 19 700 with one per cent eosinophils

The first diagnosis was pneumonia or pulmonary embolism In the afternoon of April 8 1968 she was transferred to our hospital On admission she had no subjective symptoms. A mild skin rash which had developed during her stay in the emergency department was noted The B P was 180 100 The respiratory sounds were still depressed and some rales were heard in both lung bases.

Treatment with ampicillin and digoxin was started. Next morning the skin rash had progressed severely The white cell count was 11 700 with 18 5% eosinophils Ampicillin was discontinued and she was started on tetracycline Since the skin rash had not improved on the next day prometazine 25 mg twice daily was started and in a few days the rash disappeared completely Since no further signs of cardiac decompensation or infection were noted, digoxin and tetracycline were discontinued a few days later

The chest roentgenogram one day after admission to hospital on April 9 (Fig 2 *a* and *b*) shows the rapid clearing up of the diffuse pulmonary infiltration Nine days later on April 18 the pleural effusion had also disappeared and the roentgenogram had normalized (Fig 3 *a* and *b*) The ECG was normal on April 9

Repeated urinary examinations revealed no infection. The serum creatinine was normal and no changes were found in the pyelograms

At this time the aetiology of the pulmonary infiltration was thought to be infectious since no signs of cardiac decompensation or thromboembolism were noted However one week later no certain proofs of infection had been found and we were struck by the similarity between previously reported cases of nitrofurantoin pulmonary reactions and the history of our patient She would not consent to take a challenge dose but an intracutaneous test with nitrofurantoin was positive Before the test the eosinophils in the peripheral blood had decreased to 14 but after the test they rose again to 20 % No leucocytosis was noted

The patient was discharged in excellent condition 20 days after admission She has returned to work and will visit her own doctor for check ups

Although the skin test is not conclusive we think that this test together with the whole clinical picture and history establishes the diagnosis of acute pulmonary reaction to nitrofurantoin

Case 2 H R born 1897

A 69 year-old housekeeper who had been treated for syphilis in 1928 Since 1944 she has suffered from epigastric pain occasionally combined with nausea vomiting and diarrhoea The abdominal trouble has led to several hospitalizations. In 1947 gastritis with a suspected gastric ulcer was found In 1956 a gastric ulcer was diagnosed in connection with sudden haematemesis. Gastric



Fig 2a and *b* Case 1 April 9 36 h later than Fig 1 The X ray shows the rapid improvement after discontinuing the drug

resection (Billroth II) and vagotomy were performed In the years 1956–1958 investigations were made which led to the diagnosis of cirrhosis of the liver This diagnosis proved later to be incorrect In 1961 the gall bladder was removed and in this connection biopsies of the liver showed at most a very slight subchronic inflammation of the bile ductules The abdominal troubles have continued and in 1967 chronic pancreatitis was diagnosed

In 1965 mild congestive heart failure and auricular fibrillation were diagnosed After digitalization the chest roentgenogram has been normal The auricular fibrillation has persisted

In the autumn of 1965 pyelonephritis was diagnosed A very resistant strain of E coli was cultured in the urine and the patient was treated with tetracycline Later on sulphonamides were given The patient had no nitrofurantoin at this time

In December 1966 the patient had fever dysuria and abdominal pains On January 2, 1967 a general practitioner prescribed sulphafurazole because bacteriuria was



Fig 3a and *b* Case 1 April 18 The roentgenogram is almost normal No pleural effusion detectable

Table I *Case 2 Blood picture during various periods of the case history*

Date in 1967		Hb (g/100 ml)	Leucocytes/ mm³	Neutrophils ( )	Eosinophils ( )
Jan	9	14 30	10 200		
	25	15 40	32 100		
	30		29 100	17 0	61 0
Feb	4		11 800	44 0	0 0
	10		11 200	50 5	13 5
Mar	13	14 70	9 900	53 5	11 5
Apr	4	14 60	7 700	72 0	1 0
Aug.	5	14 80	8 600	56 0	0 5
	30		20 700	71 5	13 5
Sep	4	14 20	25 400	25 0	60 5
	11	13 00	21 100	16 5	71 0
Oct	5	13 70	10 900	65 0	3 0
	12	14 70	9 900	57 5	13 5
	19	13 60	12 100	49 5	22.5
	27	13 60	5 800	69 0	2 0

found. The drug had no effect and she was remitted to the out patient department of our hospital on January 14 1967 There was growth of E. coli in the urine and treatment with nitrofurantoin was started in a dose of 50 mg three times daily On the third day from the start an urticarial reaction developed, with efflorescence all over the body The treatment was discontinued and the patches disappeared

On January 24 the patient was admitted to the hospital for further treatment She was in poor condition and had a slightly elevated temperature Here and there on the skin small reddening, rough spots were seen No peripheral oedema was noted. The pulse rate was about 60/min and the ECG showed auricular fibrillation The breathing rate was 28/min and over the basal parts of the lungs moist rales were audible The liver was palpable three centimetres below the costal arc.

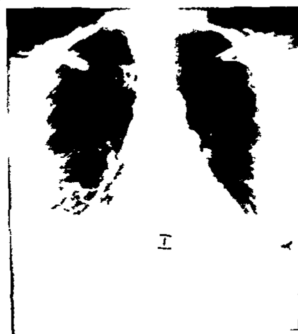


Fig 4 Case 2. Chest roentgenogram on admission on Jan 27 showing diffuse basal pulmonary infiltration. Nitrofurantoin treatment stopped seven days earlier

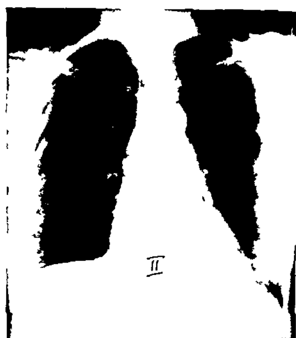


Fig 5 Case 2. Chest X ray seven days later than Fig. 4 Feb 2 showing nearly normal conditions

The blood picture on admission to the ward is seen in Table I The urinary sediment was normal and no bacterial growth was detected in culture Moderate impairment of renal function was noted creatinine clearance 64 ml min and the phenolsulphonphthalein test 49 excretion in two hours ESR was 30 mm/h Liver function tests and serum electrolytes were normal An intracutaneous test with nitrofurantoin was negative

The first chest roentgenogram (Fig 4) showed a pleural effusion on the right side and basal infiltration which was interpreted as pulmonary congestion The patient was treated with lanatosid C 0 50 mg/day and spironolactone 100 mg/day (Before admission to hospital the patient had used digoxin 0 50 mg/day) Rapid improvement was noted and a chest roentgenogram seven days later (Fig 5) showed almost normal conditions At this time there was still eosinophilia but this had decreased too Later on the eosinophilia disappeared and the patient went home in good condition on March 14 1967

On August 4 1967 the patient was admitted to the hospital because increasing dysuria and signs of impaired arterial circulation (claudication angina pectoris and vertigo) had appeared There was auricular fibrillation as previously The heart rate was about 80 min There were no signs of cardiac decompensation The



Fig 6 Case 2 Chest roentgenogram on second admission to hospital Aug 7



Fig 7 Case 2 Chest X ray on Sept 1 after treatment with nitrofurantoin for ten days

ESR was 10 mm/h Blood picture see Table I The chest roentgenogram taken at this time is seen in Fig 6 In the urine significant growth of E. coli was found

The patient was treated with sulphamethoxypyridazine and sulphamethizole (Sulfapral®) from August 4 The growth of bacteria diminished but the urine was not completely sterile On August 22 treatment with nitrofurantoin was started in a dose of 50 mg three times daily Eosinophilia rapidly developed and reached its highest level on September 11 (see Table I) The treatment was stopped on September 13

The chest roentgenogram taken on September 1 (Fig 7) showed marked impairment in relation to the roentgenogram taken on admission There were bilateral pleural effusions and basal infiltrations of congestive type The heart volume was not increased in relation to earlier investigations

When treatment with nitrofurantoin was discontinued the eosinophilia gradually diminished and was almost normal on October 5 By September 27 the chest roentgenogram (Fig 8) had already markedly normalized

At this time it seemed probable that the eosinophilia was due to the nitrofurantoin medication and a provocation test was performed Nitrofurantoin was again given for ten days (October 9 to October 19) in a dose of 50 mg three times daily There was an immediate increase of the eosinophils (see Table I) After the test dosage was stopped normal differential leucocyte counts were obtained Unfortunately no chest roentgenograms were taken during the test period

The pyelonephritis and the reaction to nitrofurantoin are only of secondary importance in the case history of this patient The predominating feature for a period of more than twenty years has been the gastro-intestinal disorder

DISCUSSION

Symptoms from the respiratory system and reactions in the pulmonary parenchyma are uncommon as side effects of drug administration However several types of reactions are known Tra

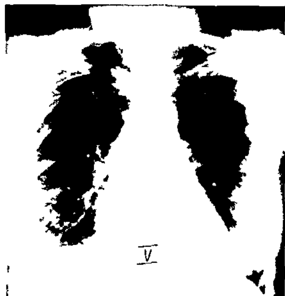


Fig 8 Case 2 Chest X ray on Sept 27 The nitrofurantoin treatment was discontinued on Sept 13 Marked improvement in relation to Fig. 7

cheobronchial airway obstruction is seen in connection with the anaphylactic reaction caused for example by penicillin Another type of reaction is the transient eosinophilic pulmonary infiltration (Loffler s syndrome) seen during treatment with para aminosalicylic acid (18) and sulphonamides (3) Furthermore pulmonary manifestations of chronic type (interstitial fibrosis) have been described after treatment with busulphan (16) and some antihypertensive drugs (5)

The pulmonary reaction to nitrofurantoin differs in many respects from the above mentioned This syndrome is best characterized as an acute febrile noncardiac pulmonary oedema as previously pointed out by Nicklaus et al (12)

Our first case follows the usual pattern described in the literature acute onset with fever dyspnoea leucocytosis eosinophilia and rapid improvement after discontinuing the drug

In the second case the symptoms appeared more slowly and the picture was confused by other diseases of the patient At the first hospitalization the patient was treated as a case of acute cardiac failure In retrospect it is difficult to decide to what extent the roentgenological pulmonary finding really was of cardiac origin Nevertheless improvement was very rapid and coincided with the stopping of the nitrofurantoin medication Consequently we suspect that the reaction was drug induced At the second hospitalization there was no cardiac decompensation and the pulmonary reaction was certainly of non cardiac origin

Since it was possible in both cases (case 2 second hospitalization) to exclude infectious pulmonary diseases thromboembolism and congestive heart failure it seems clear that the reaction was caused by nitrofurantoin This was the only drug given at the actual moment in the first case and in the second nitrofurantoin was shown by a provocation test to be the agent causing the eosinophilia No chest roentgenograms were taken during the provocation but earlier roentgenograms show that the roentgenological findings parallel the eosinophilia

The development of roentgenological signs in connection with eosinophilia and the very rapid spontaneous disappearance of the pathological signs after discontinuation of the drug support the assumption of a hypersensitive type of reaction

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INTERACTION OF HUMAN PERIPHERAL LYMPHOCYTES AND GRANULOCYTES IN THE MIGRATION INHIBITION REACTION

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Abstract The inh'bition of migration of sensitive periph eral human leucocytes has proven to be a specific parameter of cellular hypersensitivity in vitro The under lyin• mechani m is however poorly understood. The pres ent experiments indicate that sensitive leucocytes upon contact with pecific antigen i e Brucella abortus Bang, deliver a factor into the medium which is able to inhibit the migration of non sensitive leucocytes. Sens tive lym phocytes alone are not able to produce this factor In order to get an inhibition both sensitive lymphocytes and granulocytes, which need not be sens tive must be present. The possible explanations of the interaction be tween lymphocytes and granulocytes are d scussed es pecially in relation to animal experiments in which the same principal cell types seem to be invol ed.

Since the original work by Rich and Lewis (14) the inhibition of in vitro migration of various cell types by antigens has been widely used as a para meter of cellular hypersensitivity

The majority of studies has been made on animals but recent communications indicate that the method can also be applied in the human (13 17 22) These experiments are all based upon the capillary tube technique described by George and Vaughan (10) and later modified by David and coworkers (4) The inhibition of migration has proven to be a reliable parameter of cellular hy persensitivity in some of its manifestations such as brucella hypersensitivity (17) tuberculin hyper sensitivity (22) and possibly contact dermatitis (13) Various human cells have been used i e peripheral leucocytes (13 17) and lymph node cells (22) The results obtained in animal as well as human experiments seem to be comparable and there is good reason to believe that the same fundamental process is instrumental in the various experimental modifications

The mechanism of this fundamental process is broadly speaking unknown apart from some data from animal experiments indicating that the lym phocyte must be considered the immunologically specific cell in the reaction (2 7)

The purpose of the present work has been to study some of the factors inducing inhibition of migration of human cells especially as regards the interaction between lymphocytes and granulo- cytes and to try to correlate these findings with the data from animal experiments

MATERIAL AND METHODS

The material consisted of 41 brucella positive and 31 brucella-negative persons

Brucella hypersensiti ity was defined in the following way a leucocyte migration index below 0 78 at an antigen concentration of 50 mill brucella bact. per ml (see below) This has been shown to be the borderline between the brucella positive and brucella negative ob servations (18) Persons were considered brucella negati e if they did not fulfil the above ment oned criterion.

The hypersensitive group consisted of spontaneous bru cella-positive persons as well as persons vaccinated with 1000 mill killed brucella bact (Brucella abortus Bang)

Leucocyte migration inhibition studies

Migration stud es were performed with peripheral human blood leucocytes according to the technique described in detail in a previous paper (17)

In the present experiments two different cell popula tions have been used, one obtained by spontaneous sedi mentation of heparinized blood at 37 C in 45 min, the other by separation of dextran-blood on cotton wool ac cording to a technique originally described by Fichtelius (9) and modified by Hellung Larsen and Andersen (1_)

The a erage composition of the spontaneously sed mented cell population was 35% lymphocytes, 63 granulocytes and 2% monocytes In the following this

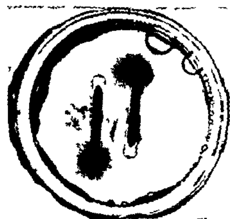


Fig 1 The arrangement of the capillary tubes in the tissue culture chamber

cell population will be referred to as leucocytes or non separated cells

The separation procedure gave the following result 97 lymphocytes and 3 granulocytes This cell population will be referred to as lymphocyte

In both cell populations there was in addition a contamination of erythrocytes which however did not interfere with the migration or the specific activity of the leucocytes

The experimental set up demanded simultaneous cultivations of two cultures in each chamber which necessitated the use of larger culture chambers dimensioned to contain 1 5 ml tissue culture medium In all experiments two capillary tubes containing the cell cultures were inserted into each chamber and fixed with the open ends in diametrically opposite directions (Fig 1) In order further to prevent contamination of cells from one culture to another a barrier of silicone wax was placed across the bottom of the tissue culture chamber thus separating the two migration areas

Various combinations of sensitive and non sensitive separated and non-separated cells were cultivated in media with or without antigen (see below) Also identical cell populations were set up with two cultures in each chamber in order to ensure identical experimental conditions

The antigen concentration was 50 mill brucella bact per ml The cell migration areas were measured after 4 hours The average migration area of the antigen-containing cultures Mx was related to the average migration area of the control cultures Mo and expressed as a migration index Mx/Mo The migration index thus expresses the inhibition induced by the antigen in such a way that the more pronounced the inhibition the lower the migration index

RESULTS

The experiments are divided into three series the first part comprising the combination of non sensitive and sensitive leucocytes the second sensitive leucocytes and sensitive lymphocytes and the third sensitive lymphocytes and non sensitive leucocytes

Table I shows the results of the first group of experiments In the first two columns are shown the migration indices of brucella positive and brucella negative cells in separate cultures According to the chosen criterion of brucella hypersensitivity all of the brucella positive migration cultures are inhibited by the antigen while the brucella negative cultures do not show any inhibition The last two columns show the migration indices from the same subjects when the brucella positive and brucella negative leucocytes are cultured in the same chamber From each combination it appears that the inhibition of migration of the brucella positive cells is roughly unchanged while the migration of the brucella negative cells is significantly inhibited compared with the migration when cultured alone ($p <$ 0 001) The degree of inhibition of the brucella negative cells is approximately the same as in the adjacent brucella positive cultures

Table II shows the situation if non separated leucocytes and lymphocytes from brucella positive persons are cultured separately or together In

Table I *Migration indices of non separated leucocytes from 18 brucella positive and 18 brucella negative persons*

Separate chambers		Identical chambers	
Brucella pos	Brucella neg	Brucella pos	Brucella neg
0 39	0 81	0 33	0 57
0 56	1 00	0 66	0 68
0 59	0 98	0 63	0 74
0 60	0 85	0 61	0 60
0 60	1 00	0 74	0 80
0 60	0 89	0 73	0 77
0 65	0 88	0 68	0 72
0 65	0 98	0 70	0 71
0 66	0 92	0 70	0 78
0 66	0 98	0 77	0 8[illegible]
0 66	0 80	0 76	0 59
0 67	0 96	0 60	0 80
0 70	0 94	0 73	0 67
0 70	0 90	0 76	0 78
0 71	1 00	0 77	0 74
0 74	0 89	0 67	0 63
0 74	0 83	0 70	0 73
0 75	0 90	0 78	0 71
Mean 0 65±0 08	0 9[illegible]±0 07	0 68±0 10	0 71±0 08

contrast to the marked inhibition of the non separated cells the migration of brucella positive lymphocytes in separate cultures is not inhibited by the antigen If however the migration of the two cell types takes place in the same chamber the migration of the lymphocytes is inhibited although not to the same degree as that of the corresponding non-separated leuco ytes The in hibition of migration of the non-separated leucocytes is unchanged whether cultured alone or together with the lymphocytes Although the inhibi tion of the lymphocytes is not very pronounced the change of migration capacity of the lymphocytes induced by the presence of a non separated leucocyte culture in the same chamber is sta tistically significant ($p < 0\ 001$)

Table III illustrates a more complex situation The first two columns show the above mentioned discrepancy between the migration of non sep arated leucocytes and lymphocytes from 13 brucella positive individuals The third column shows the migration indices of 13 brucella negative persons All cells were cultured in separate cham bers The last two columns show the result when the brucella positive lymphocytes and the brucella negative non separated leucocytes were cultured together It appears that both cell populations when cultured separately do not show any inhibi tion but when cultured together the cell migration is inhibited as illustrated by the statistically sig nificant fall of the migration indices ($p < 0\ 01$ and $p < 0\ 001$)

Table II *Migration indices of non separated leuco cytes and lymphocytes from 10 brucella positive persons*

Separate chambers		Identical chambers	
Non-sep leucocytes	Lymphocytes	Non-sep leucocytes	Lymphocytes
0 51	0 96	0 64	0 74
0 60	1 0	0 50	0 78
0 62	1 02	0 65	0 63
0 65	1 06	0 68	0 68
0 65	0 98	0 64	0 75
0 65	0 91	0 55	0 82
0 66	1 03	0 67	0 75
0 67	1 10	0 60	0 65
0 67	0 95	0 56	0 77
0 67	0 95	0 67	0 79
Mean 0 64±0 05	1 0 ±0 09	0 6?±0 06	0 74±0 06

Table III *Migration indices of brucella positive non separated leucocytes and lymphocytes and brucella negative non separated leucocytes*

Separate chambers			Identical chambers	
Brucella positive		Brucella negative	Brucella positive	Brucella negative
Non-sep leucocytes	Lympho cytes	Non-sep leucocytes	Lympho-cytes	Non sep leucocytes
0 60	1 03	1 00	0 74	0 67
0 61	1 02	0 94	0 80	0 71
0 64	0 98	0 86	0 85	0 67
0 64	0 97	0 95	0 97	0 66
0 65	1 03	0 87	0 80	0 70
0 65	1 03	1 00	0 9	0 73
0 65	1 06	0 89	0 78	0 76
0 68	1 04	0 88	1 06	0 70
0 70	0 96	0 83	0 70	0 75
0 70	0 93	0 9	0 80	0 73
0 73	1 08	0 94	0 90	0 63
0 74	1 02	1 00	0 81	0 75
0 77	1 10	1 00	0 78	0 64
Mean 0 67±0 05	1 0?±0 05	0 93±0 06	0 84±0 10	0 70±0 04

DISCUSSION

The following conclusions can be drawn from the present experiments

(*a*) Sensitive leucocytes are able to confer the migration inhibitive quality of the antigen upon non sensitive leucocytes when cultured in the same chamber This effect is present without direct contact between sensitive and non sensitive cells

(*b*) The migration of sensitive lymphocytes is not inhibited by the specific antigen when the cells are cultured alone

(*c*) The migration of sensitive lymphocytes is inhibited by the specific antigen when the lymphocytes are cultured in the same chamber as leucocytes which may be either sensitive or non sensitive

(*a*) Simultaneous cultivation of sensitive and non sensitive cells in identical chambers has pre viously been performed in animal experiments with variable results David and coworkers (5) were not able to demonstrate any effect of sen sitive peritoneal exudate cells upon non-sensitive cells in experiments with tuberculin hypersensitiv ity in guinea pigs Svejcar and coworkers (15) in similar experiments were able to demonstrate in hibition of non sensitive spleen cells in 50 % of

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INFLUENCE OF THE SITE OF MYOCARDIAL INFARCTION ON MORTALITY RATE

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Abst act The influence of the location of infarction de termined by electrocardiography on mortality in a series of 864 patients with myocardial infarction has been stu died restrospectively 183 of these patients died (21 2) 136 were autopsied and the location of the infarction was determined histologically On the basis of the ECG findings the material was divided into anteroseptal an terior posterior and indefinite groups Mortality in the indefinite group was 38 0 anteroseptal group 23 8 anterior 22.8 and posterior 14 2 In view of the autopsy results it would seem that the mortality rate for antero septal infarction is higher than that for anterior or posterior infarction Diabetes and hypertension did not in the present series affect the infarction mortality whereas bundle branch block seen in the ECG distinctly increased the mortality

The factors affecting the prognosis of acute myo cardial infarction have been very much discussed Peel et al (19) worked out a coronary prognostic index attempting to measure the relative influence of various factors on the mortality rate According to them the most potent factors increasing the death rate in the order of their severity were cardiogenic shock a history of myocardial in farctions arrhythmia bundle branch block QS complex in the ECG cardiac failure and ad vanced age Lemlich in 1965 also found that the cardiogenic shock was the greatest risk factor for patients with infarction (13) Neither of these papers devoted attention to the site of the infarc tion

Studies on the influence of the electrocardio graphic site of the infarction have usually com pared anterior and posterior wall infarctions In earlier papers a number of authors concluded that no difference was demonstrable in mortality be tween the anterior and posterior wall infarctions (14 17) Nylin et al (18) in 1943 stated that posterior wall infarction had a poorer prognosis whereas other authors have found the anterior wall infarction to be the more dangerous (4 8) Recently published papers on patients treated in coronary-care units seem to suggest that the ma jority of infarctions leading to complications were in the anterior wall region (5 12 20) In one series of 128 patients with infarction treated in the coronary care unit the mortality rate was higher in posterior wall infarctions (6) This was attributed to the bradycardia which more often accompanies a posterior than an anterior wall in farction

Septal lesion has been found dangerous (14 17 28) probably owing to lesions in the conduction system

The purpose of the present retrospective study was to ascertain whether or not the electrocardio graphic localization of myocardial infarction is of any significance for the patient s prognosis

MATERIAL AND METHODS

The series comprised all those patients treated for cardiac infarction in 1963–1966 in the Department of Medicine of Oulu University for whom a reliable 12 lead ECG was available All patients treated for infarction during th s period are not included since a complete ECG taken immediately on admission was not available in all cases particularly in 1963 and 1964 The series therefore contains no patients on whom complete ECGs could not be taken before they died. If the diagnosis was recorded with a question mark the patient was not included

The series ultimately comprised 864 patients Their sex and age distribution year by year are presented in Table I 183 patients died of the infarction while 681 were discharged as convalescents The electrocard ographic localization of the infarction was determined according to Lipman (15) by the same person in all cases To facilitate

Table I *Distribution of the series annually by age and sex*

Year	Total	Men	Women	Less than 41 y	41–45 y	46 65 y	More than 65 y
1963	167	124	43	6	9	112	40
1964	209	152	57	14	13	120	62
1965	215	156	59	12	10	137	57
1966	273	201	72	25	12	156	79
Total	864	633	231	57	44	525	238

Table II *Influence of the location of myocardial in farction determined by ECG on the mortality*

	No of cases	Deaths	Mortality ( )
Anteroseptal	202	48	23 8
Anterior	2?4	51	2? 8
Posterior	346	49	14 2
Indefinite	92	35	38 0
Total	864	183	21 2

Table III *Comparison of the sites of infarction in 136 autopsied patients as localized by electrocardiography and autopsy*

Localization by autopsy	No of cases	Localization by ECG: Antero septal	An terior	Pos terior	In defi nite
Anteroseptal	51	24	11	2	14
Anterior	37	2	29	1	5
Posterior	48	3	3	35	7
Total	136	29	43	38	26

the treatment of the material the localizations were limited to four groups anteroseptal, anterior posterior and indefinite The anteroseptal group here covers Lipman s anteroseptal and extensive anterior infarction groups The anterior covers his strictly anterior anterolateral and high anterolateral groups The posterior location corre sponds to the inferior inferior lateral posterolateral and strictly posterior groups The infarctions for which the electrocardiographic localization could not be determined were referred to the indefinite group

Autopsies had been performed on 136 of the patients who died (73 1%) The location of the infarction was assessed on the basis of both the macroscopic and the microscopic findings. The anatomical location determined from autopsy was compared with that determined from the ECG This provided evidence of the reliability of the assessment of the location from ECGs.

RESULTS

Infarctions in the anterior wall region were more common in the series than those in the posterior wall region (Table II) Anteroseptal and anterior infarctions totalled 426 while posterior totalled 346 Electrocardiographic localization proved im possible for 92 patients Table II shows further that the mortality in the total series during hospi talization was 183 patients (21 2°) In the an teroseptal group the mortality was 23 8° in the anterior 22 8°₀ and in the posterior group 14 2% The mortality was highest (38 0°₀) in the indefinite group Table III gives a comparison of the locations based on electrocardiography and those verified on autopsy The posterior and the anterior lesions were shown by the ECG with a fair degree of reliability whereas less than half of the cases of anteroseptal lesion were demonstrable from the ECG Septal infarction has proved dif ficult to localize by ECG (1)

The influence of hypertension diabetes and bundle branch block on mortality is illustrated in Table IV According to the present series neither hypertension nor diabetes noticeably affected the mortality of cardiac infarction patients The mor tality of patients with left or right bundle branch block was distinctly increased (47 1°) The in cidence of left bundle branch block was slightly higher than that of right Mortality of left bundle branch block patients was 50°₀ and of right 40 ₀

Table IV *Influence of hypertension diabetes and bundle branch block on infarction mortality*

	No of cases	Deaths	Mortality (%)
B P over 160/95 mm Hg	325	70	21 5
Diabetes	111	24	21 6
Bundle branch block	85	40	47 1
Total	864	183	21 2

Table V *Additional findings in the 92 patients whose electrocardiograms did not help to locali e the infarc tion*

B P over 160/95 mm HG	55
Age more than 65 years	40
Relapse of infarction	36
Bundle branch block	39
Infarction shock	32

Autopsy revealed 51 infarctions classified as anteroseptal 37 as anterior and 48 as posterior The same ECG localization was noted in 24 of the anteroseptal (47) 29 of the anterior (78) and 35 of the posterior infarctions (73 ⁄) Of the infarctions of the indefinite group more than half were revealed by autopsy as anteroseptal In the autopsied material there were 88 anterior wall infarctions and 48 of the posterior wall This ratio was still higher for anterior wall infarctions than the ratio from ECG (426 anterior and 346 po terior wall infarctions)

DISCUSSION

Among the factors restricting the determination of the site of myocardial infarction by ECG are several which are extracardial such as obesity or pathological changes in thoracic wall the elec trical position of the heart freshness of the in farction and the site of the infarction (transmural intramural or subendocardial) (15) As its best ECG only illustrates the electric phenomena of the heart not its anatomical state Schematic distribution of localizations into predetermined groups is not always successful since fresh in farctions are not always formed according to set patterns The infarction may comprise a part of two predetermined localizations and represent neither in pure form Furthermore there are in farctions leaving no trace on the ECG or pro ducing non specific traces (26) A material exa mined in great detail comparing the ECG find ings of 122 patients with coronary artery disease to the myocardial status seen on autopsy revealed that the ECG finding was reliable in 82° of the fresh and 27 of the old infarctions (26) Although the ECG diagnoses were not numerous nearly all showed some even if indefinite changes There was only one exception with an ECG of completely normal appearance

At autopsy of a fibrotic myocardium or an in farction less than six hours old it is difficult to say exactly where the fresh infarction lay (27)

In the present series the anterior wall infarc tion was more common than the posterior wall infarction In autopsy material the ratio was still higher for the anterior wall infarctions than in the ECG classification The proportion of antero-septal infarctions in the present ECG material is apparently too low owing to the diagnostic dif ficulties described above (1) More than half of the autopsied cases with infarction of the in definite ECG group proved to be anteroseptal or purely septal Since the mortality of the indefinite group was highest it may also be assumed that the mortality of the anteroseptal group was higher than could be proved in the present series

If the anteroseptal and anterior infarctions are combined into one anterior group the mortality in this group (23 2) exceeded that of the posterior wall infarctions group (14 2 ₒ) The present results therefore support those reported by Jacobs and Burstein (4 8)

A detailed study of the group in which the in farction could not be localized by ECG (the in definite group) reveals that 55 of the 92 patients had blood pressures exceeding 160/95 mm Hg 40 were over 65 years of age 39 had bundle branch block 36 had a relapse of the infarction and 32 had infarction shock In this group the mortality was highest 38 Some other authors have also found that mortality was highest in in farctions which in ECGs were obscure (23) It is evident that the additional findings listed above such as hypertension bundle branch block earlier infarction and shock produce ECG changes which make the localization of the infarction difficult It cannot be stated therefore that an obscure ECG finding is a sign of a dangerous infarction yet the factors responsible for the obscure ECG find ing increase mortality

Many authors report that the mortality rate for infarctions increases with age (7 19 25 26) In an infarction material of 200 patients mortality among patients under 45 was 5 in the 50–54 age group 30 ⁄ and for patients over 69 years 61 % (22) The bundle branch block patients tend to be older than the other infarction patients (2) In the present series 16° of all patients over 65 and 8 5° of patients aged 46–65 had bundle branch block but only two patients under 46

Bundle branch block is also associated with in creased mortality of infarction (2 11 16 22) Left bundle branch block has traditionally been considered more dangerous than the right (15) In the present series 50 of the patients with left and 40 of those with right bundle branch block died The difference therefore was not very great The bundle branch block is usually a sign of septal lesion a factor giving added support to the present authors view that the prognosis of

infarctions in the septal region is somewhat poorer than that of the others

According to some authors hypertension increases infarction mortality (21) while others have not found this to be the case (10) In the present series mortality of the hypertensives did not differ from that of the series at large

Coronary artery disease is known to be common in diabetics According to Biorck (3) and certain others (10 14 17) diabetes makes the prognosis of an infarction patient poorer In the present series the prognosis of diabetics did not differ from the general mortality

In the present study no attention was devoted to the extent of the infarction which naturally plays an important part in mortality Burstein (4) found from his studies that anterior wall infarctions usually were more extensive than the posterior wall infarctions This may help to explain why mortality of anterior wall infarction was higher

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RIGHT ATRIAL ECG RECORDING BY MEANS OF A SALINE FILLED POLYETHYLENE CATHETER

A bedside method useful in the analysis of cardiac arrhythmias

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Abstract Right atrial ECG recording by means of a polyethylene catheter filled with 5% saline has been performed as a bedside procedure in 100 patients in a unit for intensive coronary care It has proved a safe and useful method in the differential diagnosis of cardiac arrhythmias, providing tracings of a good quality and clear definition of atrial activity

In recent years the use of continuous electrocardiographic monitoring of patients with acute myocardial infarction has shown that cardiac arrhythmias complicating this condition are much more frequent than was originally thought For the treatment of these arrhythmias an increasing number of potent anti arrhythmic drugs is available together with instruments for electrical cardiac stimulation Exact analysis of arrhythmias is a prerequisite for the selection of proper therapy as well as for evaluation of therapeutic measures (7)

The following two problems are often encountered in the differential diagnosis of cardiac arrhythmias One concerns the recognition of the exact mechanism in clearly supraventricular tachycardias for which the conventional 12 lead ECG does not always provide sufficient information Another problem is the differentiation of tachycardias associated with abnormal QRS complexes In the presence of established intraventricular block or aberrant conduction during the tachycardia the differentiation between ventricular a–v nodal and supraventricular arrhythmias requires clear definition of atrial activity As such information is not usually provided by the conventional 12 leads special techniques must be used

In 1906 Cremer (2) introduced oesophageal electrocardiography and this method was taken up by Brown in 1936 (1) In many cases a correct electrocardiographic diagnosis could be made in this way However certain disadvantages have made other techniques desirable

Intra atrial ECG recording is another and more direct method of visualizing atrial activity and has several advantages over oesophageal leads Jewitt (5) Sivertsen (9) and Vogel (10) and their coworkers have reported their results with a platinum tipped teflon or nylon-coated steel wire Hellerstein et al in 1949 (4) and Dreifus et al in 1966 (3) used a saline filled polyethylene catheter as an electrode

In our unit for intensive coronary care we have used a modification of the method described by Dreifus for right atrial ECG recording at the bedside This method has proved a useful diagnostic aid and has the further advantage of causing no major discomfort to the patient

MATERIAL AND METHODS

From November 1966 to March 1968 about a hundred patients have had a catheter placed in their right atrium. Some of these patients were admitted to the unit because of an arrhythmia, others because they were suspected to have acute myocardial infarction.

The catheter we have used (Polystan 5 A) is a 90 cm long polyethylene tube with an inside diameter of 1 14 mm. The tube is mounted on a blunt 16-gauge needle the hub of which carries a small ring fitting exactly one end of a banana plug. Under sterile conditions a nylon guide wire with a diameter of 0 70 mm is introduced percutaneously by the Seldinger technique (8) into a suitable median antecubital vein The polyethylene cath

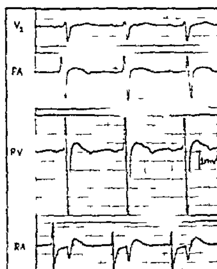


Fig 1 Normal intracavitary potentials recorded from the pulmonary artery (P A) right ventricle (R.V) and right atrium (R A) in mid atrial position as compared to V

eter is then passed into the vein over the guide wire so that the tip of the guide wire lies within the tube which is now advanced to the superior caval vein This distance must be estimated in each case by external measurement Then the guide wire is removed and the catheter is emptied of air and filled with 1 5 ml of a 5 saline solution By means of a sterile insulated extension wire with a banana plug at each end the catheter is con nected to the V lead terminal of an electrocardiograph At this point the utmost care must be taken that the electrocardiograph and the patient are properly earthed (11) Under continuous recording of the ECG the cath eter is advanced to the right atrium where the large amplitude P waves are inverted in high atrial position biphasic in mid atrial and upright in low atrial position If the tricuspid alve is passed the P waves will become quite small whereas the QRS complexes will be of very large amplitude (Fig 1) We leave the catheter in mid atrial position It is kept open by a slow intravenous

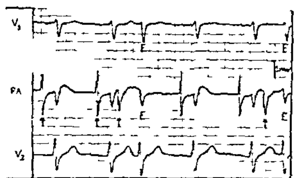


Fig 2 Atrial extrasystoles (E) Arrows point to P waves. The first two P waves are sinus P waves, the third and sixth are ectopic atrial potentials

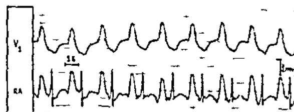


Fig 3 Ventricular tachycardia with retrograde conduc tion

glucose drip Whenever an arrhythmia occurs the cath eter is again filled with saline and the intra atrial ECG is recorded. The catheter could also be used for measur ing central venous pressure and for administration of drugs

All the ECGs presented in this paper were recorded with a Mingograph 31 B Elema Schonander We have recorded the intra atrial lead together with V and V_2. The paper speed is 50 mm/sec unless otherwise stated.

RESULTS

In the following paragraphs some of the ECGs obtained by the above mentioned method will be described and some diagnostic problems will be briefly touched on

In Fig 2 a regular sinus rhythm is seen to be interrupted by two supraventricular premature beats Atrial activity preceding these premature beats is obscured in V_1 and V whereas the intra atrial lead clearly shows two different kinds of atrial potentials The premature P waves being broad and inverted are easily distinguished from the large amplitude biphasic sinus P waves Q–P for the premature beats is 0 25 sec Taken together these findings point to a low atrial focus as the origin of the ectopic beats

The next three figures illustrate the problem of differential diagnosis of tachycardias with abnormal QRS complexes

Fig 3 shows a regular tachycardia at 180/min with broad ventricular complexes (0 15 sec) A 12 lead ECG failed to disclose any atrial activity The intra atrial lead shows large amplitude upright P waves with a Q–P of 0 16 sec P–Q is 0 22 sec Atrial tachycardia could be ruled out as it was seen that tracings at different levels in the atrium all showed an upright P wave The question remained to be settled whether this was an a–v nodal or a ventricular tachycardia As pointed out by Kistin (6) this is extremely diffi cult and not always possible Retrograde activa

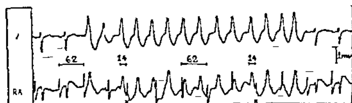


Fig 4 Short run of ventricular tachycardia with total atrioventricular dissociation Atrial capture beats indicated by *x* Paper speed 25 mm/sec

tion of the atria could be seen in both types of tachycardias A QRS to retrograde P interval of more than 0 12 sec—in this case 0 16 sec—favours the diagnosis of ventricular tachycardia

Fig 4 shows some other features which may be helpful in the differentiation between a–v nodal and ventricular tachycardia This tracing was obtained from the same patient as Fig 3 during another attack of tachycardia The rate and QRS configuration are about the same as in Fig 3 The first beat of the tachycardia is only slightly premature and is preceded by a sinus P wave Thus it probably represents a fusion beat The sixth beat may be a fusion beat too as P–Q is 0 16 sec—the same as during sinus rhythm During the arrhythmia there is mostly total a–v dissociation with P waves of the same configuration and at the same rate as during sinus rhythm On two occasions retrograde activation of the atria takes place with a QRS to retrograde P interval of 0 14 sec and upright P waves (at *x* in the figure) These atrial capture beats with a retrograde activation time of this length together with the presence of fusion beats strongly suggest a diagnosis of ventricular tachycardia (6)

Most cases of atrial flutter atrial tachycardia and sinus tachycardia with abnormal QRS complexes could be correctly diagnosed by means of an intra atrial lead Fig 5 shows a regular tachycardia at 140/min and a right bundle branch block pattern in V_1 and V None of the usual 12 leads disclosed the underlying mechanism whereas the right atrial lead clearly demonstrated an atrial flutter at 280/min with 2 1 atrioventricular block

The following three figures demonstrate atrial activity of different kinds in patients with atrioventricular conduction disturbances and rather slow ventricular rates

Fig 6 was obtained from a 19 year-old man with acute myocarditis of unknown etiology There is a regular ventricular rate of 80/min and QRS complexes as in left bundle branch block Atrial activity is obscured in V_1 but the intra atrial lead shows atrioventricular dissociation with an independent atrial activity at a slightly higher rate

A 78 year-old man was admitted because of Stokes–Adams attacks A 12 lead ECG showed a nearly regular ventricular rate of 46/min whereas no signs of atrial activity were seen An intra atrial lead showed atrial potentials of different kinds (Fig 7) In the left part of the figure there is flutter fibrillation and probably a–v dissociation (which would be seen more clearly if a longer strip could be presented) The right part of the ECG contains two biphasic sinus P waves which are possibly conducted to the ventricles though with an extremely prolonged conduction time of 0 44–0 46 sec The QRS complexes are followed by P waves of a slightly different configuration and a QRS to *P* interval of 0 18–0 20 sec This is suggestive of reciprocal activation of the atria which is possible with prolonged antegrade conduction

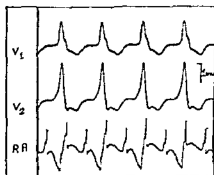


Fig 5 Atrial flutter with 2 1 atrioventricular block

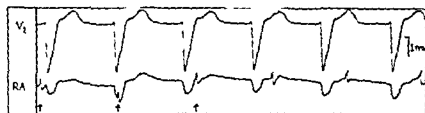


Fig. 6. Nodal rhythm with total atrioventricular dissociation. Arrows point to P waves.



Fig. 7. Bradycardia. In the left part of the ECG flutter fibrillation is seen. In the right part there is extreme prolongation of P-Q and probably reciprocal activation of the atria.

Fig. 8 is a tracing from a 75 year-old man admitted because of increasing heart failure and a slow pulse rate. It was known that he had had atrial fibrillation with a normal ventricular rate for some years. He did not receive any medical treatment. Routine ECG showed a regular ventricular rate of 43/min and a right bundle branch block pattern. No atrial activity was seen. A right atrial ECG showed flutter fibrillation, and so atrioventricular dissociation was present.

DISCUSSION

As illustrated in the preceding paragraphs special techniques are often necessary for elucidation of cardiac arrhythmias as atrial potentials may be poorly defined in a routine 12 lead ECG.

In most cases of clearly supraventricular tachycardias exact definition of atrial activity can be obtained by means of an oesophageal or right atrial lead. A more difficult problem is the differentiation of tachycardias with abnormal ventricular complexes. Even oesophageal and intra-atrial leads may fail to demonstrate the underlying mechanism. Sinus tachycardia, atrial tachycardia, and atrial flutter could nearly always be diagnosed correctly in this way even in the presence of broad QRS complexes, whereas the differentiation of a-v nodal from ventricular tachycardias is extremely difficult. In many cases only a presumptive diagnosis can be made. Retrograde conduction and total atrioventricular dissociation may be seen in both types of tachycardia. In the presence of retrograde conduction a QRS to retrograde P interval of more than 0.12 sec will favour the diagnosis of ventricular tachycardia, as will atrial captures and fusion beats when total atrioventricular dissociation is present (6).

Intra-atrial leads may also be useful in cases of atrio-ventricular or sino-atrial block and for determination of the site of origin of ectopic beats.

An oesophageal lead will often solve the above mentioned problems. On the other hand intra-atrial ECG recording has several important advantages over oesophageal leads. The procedure can be carried out without any major discomfort to the patient, and cooperation from the patient is not necessary. It often offers a better visualization of atrial activity and the tracing has a stable baseline in contrast to the wandering baseline often seen in the oesophageal leads. It is feasible in seriously ill, shocked or unconscious patients in whom the passing of an oesophageal tube will be difficult or even contraindicated.



Fig. 8. Flutter fibrillation and nodal or idioventricular rhythm. Paper speed 25 mm/sec.

The risks associated with this procedure are the same as in other situations in which a catheter is placed in the heart Atrial premature beats and fibrillation ventricular ectopics and maybe even ventricular fibrillation can be provoked mechanically with the catheter The use of a nylon guide wire for the first part of the introduction of the catheter allows the use of a very soft catheter

In our material ventricular ectopics occurred in some cases when the catheter inadvertently was advanced to the right ventricle They always disappeared after withdrawal of the catheter to the atrium Ventricular fibrillation did not occur In three cases atrial fibrillation occurred preceded by multiple atrial ectopics as the catheter entered the right atrium In one case the arrhythmia stopped spontaneously 10 minutes later In two cases the catheter was withdrawn but the arrhythmia persisted for 2–3 hours These patients had acute myocardial infarction One of them had recurrent attacks of atrial fibrillation several times later during his stay in the unit Thus the first attack need not necessarily have been caused by the introduction of the catheter

The risk of producing ventricular fibrillation due to current leakage through the saline filled catheter must also be mentioned Every possible measure should be taken to avoid this serious iatrogenic complication This means that every piece of electrical equipment connected to the patient should be correctly earthed and regularly tested for possible leakage current The risk of provoking ventricular fibrillation in this way will depend not only on the voltage source but also on the resistance of the lead system On this point the saline filled polyethylene catheter has an advantage over the platinum tipped steel wire The resistance of our catheter filled with 5° saline is about 100 000 ohms as opposed to 2–5 ohms for an ordinary electrode catheter

The 1.5 ml of 5% saline necessary for one ECG registration contain only 1 3 mMol of sodium chloride Even if several tracings are made the amount of electrolytes administered will not be of any significance but naturally the catheter must not be flushed with the 5% solution

When the catheter is left in the right atrium it can be used for intravenous infusions administration of drugs, measurement of central venous pressure and for emergency endocardial pacing as a thin electrode can easily be advanced through the catheter from the right atrium to the ventricle without fluoroscopic aid

It may be concluded that in our hands this technique for obtaining right atrial ECGs has proved a safe and helpful means of elucidating cardiac arrhythmias Right atrial leads have several advantages over oesophageal leads and the high electrical resistance of the saline filled polyethylene catheter makes it more safe than an ordinary electrode catheter

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Acta med scand Vol 185 pp 237–239 1969

THE USE OF DEXTRAN AS A DIALYSING FLUID IN PERITONEAL DIALYSIS

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Abstract With the use of 6% dextran solutions in saline as a dialysis fluid a normal effect is observed with regard to extraction of nitrogenous products The 6% dextran solution has a feeble dehydrating effect and as a result of the lack of buffers there is no correction of acidosis. The clinical use of dextran solution for dialysis is mainly restricted to diabetic patients without acidosis and uraemic patients with abnormal carbohydrate metabolism The dialysis should not be continued for long periods because of accumulation of dextran in the body The composition of a new dialysing fluid is suggested

The absorption of dextran from peritoneal washing in rabbits was studied by Schubert et al (7) who found that a small amount of dextran was absorbed into the blood

In 1965 Lami (6) working with dogs examined the serum concentrations of dextran at various times after intraperitoneal instillation of 1 g dextran per kg body weight It was found that the concentration was about 45 mg/100 ml after half an hour and reached a peak of about 600 mg/100 ml after about 24 hours It was further observed that the dogs entered a shock phase thought to represent a temporary circulatory insufficiency due to haemoconcentration It was thought that the dextran dehydrated the extracellular fluid compartment

Dextran was used as a dialysing fluid by Jirka and Kotkova (4) They compared it with a normal dialysing fluid and found that when dextran was used there was no increase in the volume of the dialysate as is obtained with glucose solution It was also shown that the protein loss in dextran electrolyte solution was less than with glucose electrolyte solution

MATERIAL

Twelve patients, eleven with defective renal function, and one with normal function, were examined Serum dextran concentrations were measured in blood samples taken before instillation of 2 l of 6% Macrodex® in saline into the peritoneum and after 30 min In five patients the serum dextran concentrations were followed for up to 24 h Samples of dialysate were taken after 30 min stay in the abdomen

METHODS

Dextran concentrations in dialysis fluid and serum were estimated by the method of Wallenius (8)

RESULT AND DISCUSSION

The concentration of dextran in the dialysing fluid fell from 60 mg/ml to 55–50 mg/ml during 30 min stay in the abdomen, and only a small proportion of the dextran was found in the blood after this period It is probable that some of the fall in dextran concentration in the dialysing fluid is a result of the dilution and coating effects and possibly uptake in other parts of the body The serum concentration rises from < 0 03 mg/ml to 0 96–3 73 mg/ml in the course of 6–24 h dialysis with Macrodex® the body receives a load of dextran which increases as long as dialysis is continued with some individual variation (Table I)

The distribution of molecular weights of dextran in Macrodex® and of the dextran found in the serum is shown in Fig 1

The shape of the curve indicates a degree of selectivity of the peritoneal membrane in that the low molecular weight dextrans are more readily absorbed

Transfer of both creatinine and potassium from the blood to the dextran dialysis fluid and the serum clearance of creatinine and potassium, are within the range found when commercial dialysis fluid is used (2) By estimations of total nitrogen

Table I *The serum concentration before and after dialysis with Macrodex® the amount left in the dialysing fluid after staying in the abdomen during 30 min and the concentration of dextran in the urine*

Patient	Diagnosis	Dialysis time and volume (h)	(l)	Dextran concentration (mg/ml) Serum before	Serum after	In the dialysate	70 000 M_w	40 000 M_n
G W 235	Diabetic + nephr chron Creat 11 3 mg	8½	14	<0 03	0 96	(41) 54 4 (44) 52 6		
F D 239	Nephr chron Creat 17 mg	17½	28	<0 03	1 07	(3) 55 6 (7) 54 8	54 100	26 00 M_w/M = ? 06
G H 243	Nephr chron Creat 6 2 mg	21½	30	<0 03	1 37	54 7 (3) 53 5 (9) 48 8 urine 0 38 (050 ml) total 0 8 g	51 500	25 800 M_w/M_n = 2,00
G W 267	Diabetic + nephr chron Creat 15 9 mg	9	18	<0 03	1 64	[a] Mean 56 1 (9 times) urine 0 61 (565 ml) total 0 8 g		
H B 268	Nephr chron Creat 6 4 mg	21½	28	<0 03	3 73	[b] Mean 55 2 (14 times) urine 13 0 (1 00 ml) total 15 6 g		

[a] Pat 267 total absorbed dextran = 19 3 g [b] Pat 268 total absorbed dextran = 166 9 g
M_w = weight average molecular weight M_n = number average molecular weight

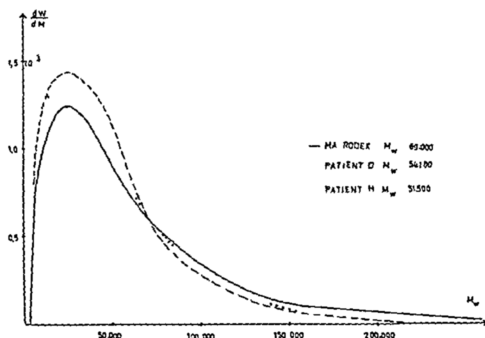


Fig 1 The distribution of molecular weights of dextran in Macrodex® and of the dextran found in the serum of two patients.

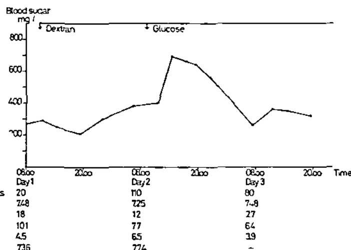


Fig ? The blood sugar curve of a diabetic patient with anuria dialysed via the peritoneum with dextran solution on the first day and with a commercial dialysis fluid on the second day The *serum creatinine* fell during both periods but an acidosis developed during dextran dialysis and an increase in body weight occurred The insulin requirement was less during dextran dialysis and increased enormously when treatment with commercial dialysis fluid was substituted associated with a fall in serum potassium

and protein in the dialysate following dialysis with dextran and commercial dialysis fluid in the same patient and temporally adjacent it was found that loss of protein is similar with the two fluids contrary to the finding of Jirka (4) that dextran has a protein sparing effect

Osmolarity of 6 % Macrodex® solution lies between 290 and 300 m osmol compared with 370 m osmol in the normal 1 ½ % glucose dialysing fluid (The glucose accounts for about 80 m osmol) Six % dextran has however a colloid osmotic pressure which gives it a small dehydrating effect during dialysis

This confirms the finding of Jirka that the dehydrating effect of dextran dialysing fluid is less than that of glucose and that an increase in body weight may accompany its use

No correction of acidosis occurs as the dextran fluid contains no buffers such as lactate or bicarbonate

The clinical use of dextran solutions for dialysis is principally for patients with diabetes without oedema or acidosis With the use of ordinary 7 % *glucose solution* in diabetics or in uraemic patients with disordered carbohydrate metabolism (1) a great increase in blood sugar occurs due to glucose absorption (3) necessitating high doses of insulin which may lead to hypokalaemia (Fig 2)

It was also shown that dextran solutions have an "anti adhesion" (5) effect in the peritoneal cavity and that pain experienced on instillation is less than with the 1 ½ % and especially the 7 % glucose solutions as a result of the relative freedom from irritation to body tissues

A dialysing fluid containing dextran sorbitol (9) electrolytes and a small quantity of glucose (to prevent its loss from the body) might prove to be more satisfactory than fluids containing dextran or glucose alone owing to lack of irritation and ease of preparation and freedom from side effects

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Congress Announcements

Universite de Paris Faculte de Medecine *Les Journees Medicales Annuelles* de l Hopital Broussais La Charite sous la presidence du Professeur Pasteur Vallery Radot de l Academie Française Service du Professeur Paul Milliez auront lieu mercredi 30 avril jeudie 1er mai et vendredi 2 mai 1969

Il est recommande de s inscrire assez tot le nombre des participants etant limite Priere d en voyer les droits d inscription au Centre de Recherches sur l Hypertension Arterielle Professeur Milliez Hopital Broussais 96 rue Didot Paris 14 (cheque bancaire ou mandat-carte) Les droits d inscription sont de 100 F tout compris (ensemble de ces journees et volume des conferences) Un fichet de reduction SNCF sera adresse sur demande

Clinique Nephrologique Hopital Necker Professeur J Hamburger 149 rue de Sevres Paris 15 *Cours de Perfectionnement sur la Nephrologie* les samedi 3 dimanche 4 et lundi 5 mai 1969

Il est recommande de s inscrire assez a l avance le nombre des participants etant limite Pour tous renseignements s adresser au secrétariat du Professeur J Crosnier Hopital Necker 149 rue de Sevres Paris 15

The Third Congress of the European Thyroid Association (Association Europeenne de Recherches sur la Glande Thyroide) will be held in Athens Greece May 27 to 30 1969

Secretary Dr C Beckers University Louvain Cliniques Universitaires St Pierre 69 Brusselstraat Louvain Belgium

The Third International Conference on Congenital Malformations will be held in Netherlands Congress Centre The Hague September 7 to 13 1969 General theme *Birth defects—1969*

Secretariat before and after the Conference Holland Organizing Centre 16 Lang Voorhout The Hague

Secretariat during the Conference Netherlands Congress Centre 10 Churchillplein The Hague

An IAEA SYMPOSIUM *In vitro Procedures with Radioisotopes in Clinical Medicine and Research* will be held in Vienna September 8 to 12 1969

Organizers International Atomic Energy Agency Karntnerring 11–13 1010 Vienna Austria

Further information and forms to accompany abstracts of papers intended for presentation at the Symposium may be obtained from national authorities for atomic energy matters Abstracts must be submitted through these authorities so as to reach the International Atomic Energy Agency before May 1 1969

The International Symposium on the Pathogeny of Epilepsy will be held in Varna October 7 to 8 1969

Address Epilepsy Research Group Sofia 31 Bulgaria

Organizing Committee Academ G Usunoff President Dr E Atsev Secretary Dr D Tchavdarov Treasurer

The Sixth International Tissue Research Conference entitled BLOOD CELLS AS A TISSUE will be held at the Lankenau Hospital Philadelphia Pa October 30 to 31 1969

Topics Regulatory mechanisms Metabolism and function of normal and abnormal cells Recent developments in therapy

Information William L Holmes Ph D Division of Research Lankenau Hospital Lancaster and City Line Avenues Philadelphia Pa 19151

Acta med scand Vol 185 pp 241–251 1969

MYOCARDIAL INFARCTION IN EARLY AGE

III *Coronary Risk Factors and Their Deficient Control*

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Abstract Two hundred and thirty survivors among 481 males, suffering their first myocardial infarction at or below the age of 50 were submitted to a thorough follow-up examination 1–18 years after their first attack Coronary risk factors as earlier established from clinical work and prospective studies were extremely prevalent as judged by comparison with a population study of 855 fifty year-old males from the same city using the same methods Serum cholesterol serum triglyceride serum uric acid, serum fibrinogen, fasting blood sugar systolic and diastolic blood pressures all showed a heavy accumulation within the higher decentiles of the population study This was especially striking as regards serum cholesterol 97.5 per cent had been smokers at the time of the attack as compared with 56 2 in the population study Efforts aimed at control of the risk factors had in the vast majority of cases been neglected or unsystematically or badly pursued

The main results of long term prospective studies of cardiovascular conditions have been the establishment of a number of factors which singly or in combination carry a considerably increased statistical risk of subsequent coronary incidents and as has been recently demonstrated also cerebrovascular lesions (14) This can hardly be considered sensational Any clinician might at least 25 years ago have firmly declared on less sophisticated and statistically backed grounds that high blood pressure elevated serum lipids diabetes—even mild—and gout were all associated with increased risks of cardiovascular disease However the realization that the risks are gradually increasing throughout the entire range of the parameters measured such as blood pressure or serum cholesterol seems to some extent to represent new thinking That latent diabetes which can only be defined by the outcome of a glucose tolerance test is also associated with an increased frequency of latent cardiovascular symptoms seems important The rather striking data of the association between particularly excessive cigarette smoking and coronary events would also seem to represent new knowledge The different degrees of physical activity as in the well known studies on London transport workers also seem to be of considerable interest

To obtain information concerning the frequency and distribution of recognized coronary risk factors in survivors after first myocardial infarction in early age groups we have collected a material from four medical departments (from Sahlgren s Hospital Medical Department I 1948–1965 Department II 1954–1965 Department III 1959–1965 and from the Medical Department Molndal s Hospital 1954–1965)

The survivors were submitted to an extensive follow up examination which combined with a scrutiny of the original records permitted an evaluation of whether efforts had been made to trace pathogenetic factors and if such were found to control them with any tenacity of purpose

The aim of the present paper then is to concentrate on these aspects and only briefly to touch upon the complicated problems of the interrelations between the risk factors which will be the main subject of future studies The relations of these factors to high and low risk occupations will be discussed in a separate communication Convenient background data have been provided from a population study on 50 year-old males from Goteborg (24) the serum measurements used in the present paper were performed in the same laboratory

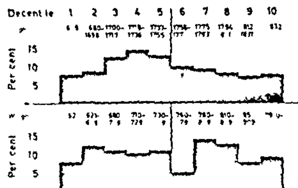


Fig 1 Percentage distribution of height and weight in survivors after first myocardial infarction ≤50 within decentiles of population group Myocardial survivors n = 230 population group n = 855

CLINICAL MATERIAL

The diagnostic criteria were a first myocardial infarction characterized by severe chest pain suggestive of infarction and ECG changes (pathologic Q waves and/or development of S T and T-changes) at an age of 50 years or below If there were any doubts about the interpretation of the ECG findings we have considered the finding of a complete occlusion on subsequent coronary angiography to be supportive if a severe chest pain had been present During the last 5 6 years this might have led to a slight widening of the diagnosis

Thus defined the clinical material included 481 males One hundred and ninety three had died 29 were from foreign countries or remote areas Of 259 living within the uptake area 29 did not participate for various reasons Two hundred and thirty (89%) underwent the follow up examination The 29 subjects not participating in the follow up study did not reveal any special characteristics of the group as regards occupation blood chemistry blood pressure diabetes etc according to the case records In 12 individuals of this group previous cholesterol measurements were available Eight of these fell within the ninth or tenth decentiles of the population group The present paper concentrates on the results of the follow up examination

The age distribution of the studied subjects when they had their first myocardial infarction was skewed to the low values Ten of them were below 39 years The median value was 47 years According to the rules of selection no one was more than 50 years of age

No difference in age distribution was found between the subjects studied at follow up and the total series of 481 subjects. The age distribution when the subjects were examined was approximately normal The median value was 53 years with 80% between 44–60 years

Population study group

Eight hundred and fifty five randomly selected 50-year old male subjects from Göteborg were investigated during 1963 A full description of the methods of selection and work up of the population study has been given in the study by Tibblin (24)

METHODS

The clinical and chemical methods used have been given in a previous paper (12)

The glucose oxidase method used in the population study however was that described by Keston (17) and Teller (23) The de Verdier (25) modification of this method which was used in the study of survivors of myocardial infarction yielded on an average 6 mg lower values in determinations on 100 blood samples This difference diminished at the higher levels of the normal range The correction used (6 mg) has been omitted at values above 100 mg%

Glucose tolerance tests nephelometry and ultracentrifugal separation were not used in the population study The findings by the two latter methods will be given in another connection

RESULTS

(Distribution of weight height serum cholesterol serum triglyceride serum uric acid systolic and diastolic blood pressure and blood sugar given within respective decentiles of the population study The results have been given in Tables I–VIII and Figs 1–3)

Weight and height

From Fig 1 it is apparent that the weight of the myocardial survivors was evenly distributed within the decentiles of the population study There was however a tendency for the myocardial survivors to aggregate within decentiles 3 4 and 5 as regards height leading to the suggestion as pointed out by others (7) of a tendency for myocardial infarction in early age groups to occur more frequently in short heavily built males More detailed information on height-weight ratios in relation to serum lipids glucose tolerance and serum uric acid will be given in another paper

Serum cholesterol and serum triglyceride

Table I and Fig 2 contain data of serum lipid levels The grouping as to dietary habits has been performed as follows

No diet—no dietary restrictions whatsoever

Diet—avoidance of visible fat—pork bacon ham cream and butter Restriction of eggs Use of oleomargarine moderately high content of polyunsaturated fat (25%)

Strict diet—same as above Also consistent use of highly polyunsaturated oils (corn oil sunflower oil etc) skimmed milk fish at least three times

Table I *Percentage distribution of serum cholesterol and serum triglyceride in survivors after first myocardial infarction ≤50 years within decentiles of population study of males at 50 years*[a]

No diet $n=81$ diet $n=90$ strict diet $n=59$ lipid reducing agents $n=29$ population study $n=855$

Decentile	1	2	3	4	5	6	7	8	9	10
Serum ch lesterol mg										
	-193	194-214	215-223	224-232	233-244	245-255	256-267	68 81	282-304	305-
No d et	0 0	1 3	2 5	1 3	6 3	11 4	6 3	11 4	29 1	30 4 (3 7)
Diet	2 2	4 4	2 2	1 1	2 2 (1 1)	7 6	10 9	17 3 (1 1)	18 5 (3 3)	33 7 (2)
Strict diet	5 0 (3 5)	1 7 (1 7)	1 7	3 3 (1 7)	3 3 (1 7)	5 0	10 0 (3 5)	10 0 (1 7)	35 0 (8 8)	25 0 (10 5)
of total	2 2	2 6	2 2	1 7	3 9	8 2	9 0	13 3	26 4	30 3
Se um tr glyceride mg										
	-59	60-68	69-77	78-85	86-94	95-102	103 120	121-137	138 171	17
No diet	5 1	2 6	5 1	3 9	9 0	7 7	12 8	14 1	18 0	21 8
Diet	4 6	5 8	3 5 (1 1)	10 3 (1 1)	5 8 (1 1)	5 8 (1 1)	18 4 (2 2)	8 1	19 5 (1 1)	18 4
Strict d et	10 1 (1 7)	8 5 (1 7)	8 5	5 1 (1 7)	8 5 (1 7)	8 5 (5)	11 9 (6 9)	8 5 (3 5)	13 6 (1 7)	17 0 (8 6)
of total	6 3	5 4	5 4	6 7	7 6	7 1	14 7	10 3	17 4	19 2

Figures within brackets — on l pid reducing agents predominantly Atromid S

a week oleomargarine with a high content of polyunsaturated fat (45%) Records should be available as to the degree of adherence throughout the treatment

From Table I it is apparent that whereas very few serum cholesterol values fell within the five lower decentiles in the non diet group there were somewhat more with low or moderately low serum triglyceride levels There were a few more cases of myocardial survivors within the low decentiles in the diet and strict diet groups with or without lipid reducing ag nts (figures within brackets) Although diet and/or lipid reducing agents were used a considerable number of cases appear in the highest decentiles The average response in those submitted to lipid reducing measures where previous data were sufficient for assessment has been given in Table II In trying to trace earlier serum lipid observations it became evident that these had been very unsystematically performed Although a number of papers have appeared showing the drastic reversible early changes in opposite directions of cholesterol and triglyceride levels after myocardial infarction the majority of determinations had been made during this period or after some attempts at lipid reduction Judging from the instances in which observations were available before the infarction the serum cholesterol depression on the first day after attack was usually mild For the comparison in Table II therefore we have used values be for infarction first day values or values after

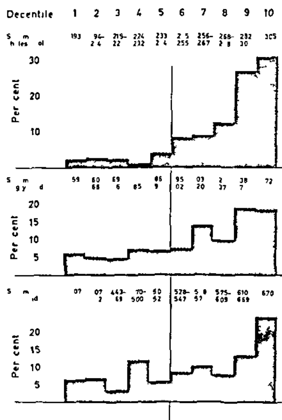


Fig 2 Percentage distribut on of serum cholesterol serum triglyceride and serum uric acid in survivors after first myocardial nfarct on ≤50 within decentiles of population group Serum parameters gi en irrespective of whether the subjects were untreated or treated with diet of drugs which m ght affect the levels.

Table II *Comparison between highest serum cholesterol recorded and serum cholesterol at follow up*[a]

	n	Mean difference (mg%)
Diet	20	49
Strict diet	33	63
Subjects within decentiles 1–5 at follow up		
No diet	4	24
Diet	8	43
Strict diet	5	74

[a] Relates exclusively to subjects with data available before first day after and three months after infarction

three months and used the highest value recorded

If we assume the figures in Table II to be representative of the whole groups on diet and strict diet this may possibly indicate that 40% (diet) of the subjects might have shifted 2–4 decentiles to the left and about 25% (strict diet) 4–6 decentiles In a few cases on strict diet and Atromid S the shift in both serum lipids had been from far to the right within the tenth decentile to the first

In Fig 2 it is apparent that although a rather strict diet had been applied in 25% some dietary restriction in a further 40% and lipid reducing s in 12.5% of those on diet there was a lure to shift the whole group of myocardial survivors to within the lower decentiles of the population group This particularly applied to serum cholesterol

Systolic and diastolic blood pressures

Those on active antihypertensive treatment were distributed fairly equally within the different dietary groups for which reason the data have been summarized in Fig 3

The accumulation of cases within the eight decentiles has tentatively been assumed partly to reflect observer bias of the type discussed by Humerfelt (13) On the whole there was a moderate shift to the right although not nearly as striking as in the case of serum lipids There was a low frequency of patients within the two lowest decentiles both as regards systolic and diastolic pressures Of the two cases falling in the lowest decentile as regards diastolic pressure one was on active treatment and one had syphilis and aortic insufficiency (the only syphilitic encountered in the follow up examination) It is also evident that active antihypertensive treatment played a negligible role in shifting the myocardial survivors to the left part of the diagram

Serum uric acid

As there was no systematic difference in the distribution of serum uric acid levels between the different dietary groups the summarized figures have been given in Table III Subjects on drugs known to raise or lower serum uric acid levels have been listed separately It is evident that whereas the myocardial survivors were distributed fairly evenly within the first nine decentiles there was a definite accumulation of cases within the tenth decentile However ten of these were on saluretics

Table III *Percentage distribution of serum uric acid in survivors after first myocardial infarction <50 years within respective decentiles of population group n = 220*

Population group *n* 839

Decentile	1	2	3	4	5	6	7	8	9	10
Uric acid level mg%	<4.07	4.07–4.42	4.43–4.69	4.70–5.00	5.01 5.27	5.28 5.47	5.48 5.74	5.75 6.09	6.10–6.69	>6.7
Per cent										
No drugs	4.5	6.8	3.6	10.7	5	8.2	9.5	5.7	11.8	18.7
On saluretics	0.5	0.5	0	1.4	0	0	0.5	0.5	0.5	4.5
On uric acid lowering drugs (Atromid S Probenecid)	0.5	0	0	0	1	0.5	0.5	2	1.5	1.5
of total	5.5	7.3	3.6	12.1	6	8.7	10.5	8.4	13.8	4.7
	34.5					66.1				

The association of gout with cardiovascular disease, the earlier findings of elevated uric acid levels in myocardial survivors and the findings of higher levels of serum uric acid in cases of hypertension with occlusive vascular disease than in those without (1, 9) are of considerable interest. However, these studies as well as the reports of statistical association between elevated serum uric acid levels and subsequent coronary incidents in some prospective studies (6, 7) are not easy to evaluate. Whether serum uric acid operates as a risk factor per se or only by way of its association with hypertensive vascular disease, hyperglyceridemia and latent diabetes will be discussed in a separate communication.

Fasting blood sugar and glucose tolerance tests

From Table IV it is evident that the slight shift to the right—56.4%, within the 5 higher decentiles—was entirely due to the non-diet group, in which 69.5% fell within the 5 higher decentiles. In the diet and strict diet groups the corresponding figures were 50 and 51% respectively. However, there seems to be some tendency to aggregation of subjects within the tenth decentile. Of the 41 subjects within the tenth decentile five were known diabetics on treatment. Ten further cases were on saluretics, which was more than half of the patients on saluretics—19.

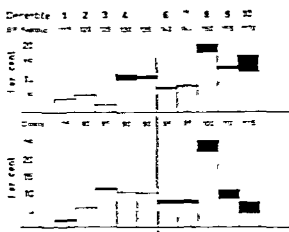


Fig. 3. Percentage distribution of systolic and diastolic blood pressure in survivors after first myocardial infarction ≤ 50 within decentiles of population group. Black part of columns—subjects on antihypertensive treatment—mainly saluretics.

Glucose tolerance tests were performed on 219 subjects. Using the criteria adopted in the study by Keen et al. (16) of a 120 minutes glucose value of 120 mg% or above we found 21 subjects with abnormal response. Of the 21 abnormal responses seven were found in the 16 tolerance tests made on subjects on saluretics. This would give a figure of —%, while the subjects not on saluretics exhib-

Table IV. *Percentage distribution of fasting blood sugar in survivors after first myocardial infarction ≤ 50 within decentiles of population group*

Myocardial survivors n = 227. Population group n = 3[illegible]

Decentile	1	2	3	4	5	6	7	8	9	10
Fasting blood sugar mg%	< 67	68–72	73–76	77–79	80–82	83–84	85–88	89–91	92–98	99
No diet, %	2.5	5.0	11.0	6.5	6.5	9.0	19.0	6.[illegible]	13.0	12.[illegible]
Manifest diabetes	0	0	0	0	0	0	0	0	0	3.9
On saluretics	0	0	0	0	0	1.1	0	0	0	[illegible]
Sum	2.5	[illegible]	11.0	6.5	6.5	12.1	19.0	6.[illegible]	13.0	19.2
Diet, %	11.1	8.5	12.0	8.9	8.5	1.1	[illegible]	8.9	[illegible]	14.5
Manifest diabetes	0	0	0	0	0	0	0	0	0	[illegible]
On saluretics	0	0	1.1	0	1.1	1.1	1.1	0	0	3.3
Sum	11.1	8.9	11.1	8.9	12.0	[illegible]	6.[illegible]	8.9	12.[illegible]	[illegible]
Strict diet, %	5.1	[illegible]	17.0	6.8	8.5	5.1	13.5	6.8	6.8	8.5
Manifest diabetes	0	0	0	0	0	0	0	0	0	0
On saluretics	0	0	0	0	1.7	0	1.7	1.7	1.7	[illegible]
Sum	[illegible]	[illegible]	17.0	6.8	10.2	5.1	15.2	8.5	8.5	13.6
Total, %	6.6	[illegible]	12.8	7.5	8.8	[illegible]	13.2	7.9	11.5	11.2
	43.6					56.4*				

Table II *Comparison between highest serum cholesterol recorded and serum cholesterol at follow up*[a]

	n	Mean difference (mg%)
D et	20	49
Strict diet	33	63
Subjects within decentiles 1 5 at follow up		
No diet	4	ˀ4
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Relates exclusively to subjects with data available before first day after and three months after infarction

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Table III *Percentage distribution of serum uric acid in survivors after first myocardial infarction < 50 years within respective decentiles of population group n = 2?0*

Population group *n* 839

Decentile	1		3	4	5	6	7	8	9	10
Uric acid level mg%	< 4 07	4 07–4 4	4 43–4 69	4 70–5 00	5 01 5 .7	5 28 5 47	5 48–5 74	5 75 6 09	6 10–6 69	> 6 7
Per cent										
No drugs	4 5	6 8	3 6	10 7	5	8 2	9 5	5 7	11 8	18 7
On saluretics	0 5	0 5	0	1 4	0	0	0 5	0 5	0 5	4 5
On uric acid lowering drugs (Atromid S Probenecid)	0 5	0	0	0	1	0 5	0 5	2	1 5	1 5
of total	5 5	7 3	3 6	12.1	6	8 7	10 5	8 4	13 8	4 7
	34 5					66 1				

Table VII *Smoking habits and type of supervision in survivors after first myocardial infarction ≤50*

	Never smoked	Stopped[a] before infarction	Stopped at or after infarction	Cigarettes <15	Cigarettes >15	Total cigarette smokers	Pipe[b]	Total
A Percentage distribution compared with population group								
Survivors after first myocardial infarction $n=230$	1 75	1 75	29 1	28 3	22 6	80 0	16 5	
Population group $n=852$	24 2	19 7	—	27 4	20 0	47 4	8 8	
B Type of supervision								
Cardiologists	—	1	9	2	4		1	17
Specialists internal medicine	3	—	19	9	10		16	57
Specialists in metabolism	1	—	19	15	12		12	59
Practitioner	—	1	4	7	3		—	15
Anticoagulant clinic	—	1	1	8	5		—	15
No physician	—	1	15	24	18		9	67
Total	4	4	67	65	52		38	230

[a] Stopped several years before infarction [b] Data incomplete regarding change from cigarettes to pipe after infarction

approach to the problems of treatment and that a good number of patients were entirely without any control

It would seem as if cardiologists specialists of internal medicine and specialists in metabolism to some extent succeeded in making a fair number of the subjects stop smoking or change to pipe smoking while in the group supervised by practitioners or attending the anticoagulant clinic the proportion of those who had stopped smoking was not higher than of those without any supervision whatsoever

Diet and drugs and medical supervision

Table VIII shows that diet as might be expected was most strictly enforced by specialists interested in metabolism but was also used in a considerable proportion of the cases supervised by

Table VIII *Type of supervision diet and drugs in survivors after first myocardial infarction <50 N=230*

	Cardiologists	Specialists internal medicine	Specialists in metabolism	Practitioner	Anticoagulant clinic	No physician	Total
No diet							
No drugs	3	6	4	4	—	37	54
Lipid reducing agents	—	1	1	—	—	—	2
Antihypertensive agents	1	5	—	—	—	—	8
Anticoagulants	1	3	2	—	9	—	15
Sum	5	15	7	6	9	37	79
Diet							
No drugs	5	21	4	1	—	25	56
Lipid reducing agents	—	1	4	1	—	—	6
Antihypertensive agents	1	5	7	1	—	—	14
Anticoagulants	—	6	1	2	6	—	15
Sum	6	33	16	5	6	25	91
Strict diet							
No drugs	5	4	4	3	—	5	21
Lipid reducing agents	—	2	18	—	—	—	20
Antihypertensive agents	1	1	6	1	—	—	9
Anticoagulants	—	2	8	—	—	—	10
Sum	6	9	36	4	0	5	60
Total	17	57	59	15	15	67	230

specialists of internal medicine and in the small group supervised by cardiologists Diet was apparently just as much used by those not under any supervision as by those under the control of practitioners or the anticoagulant clinic Surprisingly enough there were five subjects in the group without supervision who met the criteria for a strict diet

Lipid reducing agents were to an important degree used only by the specialists interested in *metabolism With the exception of a single case* on anticoagulant neither such drugs nor lipid reducing agents were used by the cardiologists

COMMENTS

This series of subjects with myocardial infarction is selected for comparison with cases classified as coronary artery disease in prospective studies It does not include sudden death during the acute course of myocardial infarction or within one year after the infarction The importance of this mechanism of selection is difficult to evaluate from the present study

The factors which for many decades have been known to be associated with clinical complications of atherosclerosis at a premature age elevated serum cholesterol elevated blood pressure and high uric acid levels were in the myocardial survivors all more or less heavily accumulated within the higher decentiles of the [illegible]lation group in the present study This [illegible]ally applied to serum cholesterol As regards the serum lipids this accumulation is considerably *more impressive as 25% adhered to a* strict diet and 40 kept some diet which in those for whom data are available had produced a rather marked lowering of serum cholesterol Insufficient data were available on previous serum triglyceride

The above factors as well as cigarette smoking have in a number of large prospective studies been shown to be more or less consistently associated with a stronely increased risk of subsequent development of coronary disease (3 4 8 15 18–21 27) As regards serum triglyceride a number of clinical studies suggest it to be of considerable importance The prospective evidence is however still rather meagre—as discussed by Brown et al (2) This would seem also to apply to subjects with latent diabetes as defined by abnormal glucose tolerance tests

In the present study practically all the subjects smoked before the attack and although 29% had stopped after the attack there were still more smokers than in the population group

The results of the fasting glucose measurements and the glucose tolerance tests are somewhat difficult to interpret The mean 120-minute glucose values increased with the time elapsing from the attack On the other hand the death records seem to show that a late follow up might *underestimate the importance of manifest* diabetes The high frequency of abnormal oral glucose tolerance tests in a number of studies of cardiovascular disease reviewed by Wahlberg (26) and found by him also with the i v glucose tolerance test contrasts somewhat with our findings This may be partly explained by age differences Some of the reports do not state whether the subjects were on saluretics The technique used by Keen (16) and by us is now being compared with the i v tolerance test in our laboratory

In a large number of subjects many of the risk factors were present in the same individual to a sometimes appalling degree The perusal of some 700–800 case records from which the present initial material of 481 myocardial infarctions was derived and of some 5000 records from which a material of about 700 cases of hyperlipidemic states have been collected has left us with a very strong impression of the general lack of interest and system in attempts to trace background factors of proven or possible pathogenetic *interest for atherosclerotic complications A single* serum cholesterol measurement sampled in the immediate myocardial period (during which a depression of serum cholesterol by more than 60% may occur) has evidently in a large number of subjects been thought to be sufficient From our study it is also apparent that the myocardial survivors to a large extent were without any supervision at all or were treated by physicians with different attitudes There was very little sign of a consistent policy being applied to the majority of the subjects

From these considerations one might advocate the policy of a more intense search for previous data coupled with a thorough examination about three months after the attack (when the serum cholesterol usually has reverted to its original level and the triglyceride elevation has almost

but not completely subsided) Such an examination should at least include repeated measurements of blood pressure serum cholesterol serum triglyceride and serum uric acid and a simple glucose tolerance test Smoking habits should be thoroughly penetrated This should be combined with tests for physical performance in preparation for an active training program

Myocardial infarction is regarded as having a multifactorial background Also in the present study there were only a limited number of cases showing a clear dominance of a single factor When this was so it was practically always a very marked elevation of serum cholesterol

There is felt to be a definite need for strictly randomized fully controlled studies using double blind placebo techniques directed against single factors such as hyperlipidmic states blood pressure carbohydrate metabolism deviations and so forth However the size of the materials and the length of the period of observation necessary until complete answers on the value of individual measures might be forthcoming seem to be formidable obstacles Other approaches seem also to be possible Another entirely rational attitude in our opinion might be the following Multifactorial background necessitates a multifactorial approach Rapid extension of knowledge improvements in drug therapy and increased realization of delayed side actions of now prevalent drugs would favour a flexible approach The main aim to keep in sight all the time should be to control as many risk factors as possible and as well as possible The limits here would be set by psychological and economical considerations

The possible yardstick for the appraisal of the efficiency of such a complex therapeutic policy might be the analysis of the survival rates of consecutive groups of myocardial survivors before and after such a policy was generally agreed upon This has been discussed in an earlier paper (11) We have earlier made some attempts at this in actively treated hypertensive disease (10) The objections that the results of such a complex approach might be obscured by possible spontaneous changes in the natural history of patients hospitalized for severe hypertensive disease and myocardial infarction seem to us not particularly valid

The concentration in the present paper on secondary prevention after the first myocardial infarction does not mean that we are not greatly in favour of the primary prevention measures eloquently advocated by among others Stamler (22)

The multifactorial approach is however at present at best a rather clumsy and often necessarily poly pharmaceutical one The study of the interrelations of coronary risk factors must be further deepened The search for ultimate biochemical mechanisms has undergone a tremendous expansion during the last 15 years and is being pursued vigorously at many centers It is a simple truism to state that resources should not be spared in this search However knowledge and techniques seem now to have advanced to a point where energetic measures in the meantime should be applied to those who have suffered a myocardial infarction at an early age or those who are known to harbour factors which seriously increase the risk

CONCLUSIONS

1 Two hundred and thirty male survivors after a first myocardial infarction ≤ 50 years were submitted to a thorough follow up examination 1–18 years after their infarction The data have been compared with data from the same laboratory obtained from a population study of 855 exactly 50 year-old males from Goteborg as regards all parameters

2 While weight was rather evenly distributed there was as regards height an accumulation of myocardial survivors in decentiles 2 3 and 4 of the population group This would mean an excess of moderately short men

3 Serum cholesterol serum triglyceride serum uric acid systolic and diastolic blood pressures were all more or less heavily accumulated within the higher decentiles of the population study

4 In the non-diet group there were only a few subjects with serum cholesterol below the median In the two dietary groups (diet and strict diet) a few more subjects were found below the median Where data were available most of these subjects had had definite hypercholesterolemia and would have originally belonged to the tenth decentile of the population study

5 Fourteen % of the myocardial survivors were under some form of antihypertensive treatment This seems only to have affected the distri

bution as regards blood pressure to a very limited extent (Fig 3)

6 Fasting blood sugar in the myocardial survivors was shifted to the right as compared with the population study only in the non-diet group while the groups on diet as well as on strict diet were equally divided above and below the median The importance of saluretics in causing elevated fasting blood sugar and abnormal tolerance tests has been emphasized

7 Ninety six and a half % of the myocardial survivors were smokers before the attack as opposed to 56.2% in the population group Twenty nine % had stopped after the attack and some had gone over to pipe smoking There seemed to be some difference in the numb r of those who had stopped according to the type of supervising physician

8 A scrutiny of the original case records revealed in the great majority of cases a severe lack of consistency and purposeful planning in attempts to trace possible or proven factors of pathogenetic importance

9 Nearly one-third of the myocardial survivors were not under the supervision of any physician The rest were treated by physicians with somewhat different attitudes to therapeutic possibilities.

10 A more decisive planning has been advocated, including a penetrating search for coronary risk factors at a suitable time after the attack i e approximately three months) and a flexible proach, the chief aim being the fullest possible control of recognized risk factors within the limits set by psychological and economical considerations and some common sense An appraisal of results is considered to be possible by making a running analysis of survival rates of consecutive groups of myocardial survivors before and after major changes in diagnostic and therapeutic policy

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Acta med scand Vol 185 pp 253–258 1969

THE PROGNOSIS IN LONG TERM TREATED AND UNTREATED ESSENTIAL HYPERTENSION

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Abstract Since 1955 we have registered patients suffering from essential hypertension 433 cases 225 men and 208 women, were graded according to WHO and Hammarstrom and Bechgaard s system. Medical treatment was started and followed up by one of us (HSM) The numbers of deaths in the treated sample and in an "untreated" series of 290 hypertensive patients are compared with the death rate in the corresponding general population in Norway The same persons have made the classification of the treated and "untreated" samples

In the treated series the numbers of deaths were 56 men and 29 women instead of the 15 61 men and 12.73 women expected which shows a death rate of 359% for men and 228% for women. This is far better than of the "untreated" sample in which the death rates were found to be 985% for men and 813 % for women It seems as if we have succeeded in reducing the death rate to about half by means of medical treatment There is an appreciable reduction in the number of cerebral deaths in men in the treated sample as compared to the "untreated" There is not a similar drop in cardiovascular deaths

The consequences of high blood pressure still represent one of the most important causes of death The beneficial effect of antihypertensive treatment on the prognosis of hypertensive patients is clear in the case of malignant hypertension (16) However there is still a lack of information concerning the effect of antihypertensive treatment on prognosis in the less severe forms of hypertension Such information should ideally come from studies of simultaneously treated and control patients As no ideal control series has yet been reported it seems justifiable to compare a series of treated patients with a series of untreated drawn from the same population and investigated and classified in a similar manner

In this paper mortality rates and causes of death in a series of treated hypertensive patients are compared with those in a series of "untreated" hypertensive patients The "untreated" material was collected in 1944 and followed up at intervals (11 12 13) The treated material consists of patients who started treatment in the years 1955–1965 and the present report concerns their progress up to January 1967

The clinical course in essential hypertension is very variable Volhard and Fahr (18) distinguished between "Gutartige essentielle Hypertonie" and "Maligne Form der Hypertonie" Levy et al (9) described two forms of hypertension

1 Transient arterial hypertension with blood pressure which fell below 150/90 mm Hg when the patient was kept in bed or on sedatives

2 Sustained hypertension with blood pressure always above 150/90 mm Hg

Keith et al (7) have on the basis of changes in the eyegrounds classified essential hypertension into four types which show different prognoses

Rasmussen (15) recommended a classification into three grades

1 Hypertensio arterialis levis
2 Hypertensio arterialis gravis
3 Hypertensio arterialis maligna

Hammarstrom and Bechgaard (3) have used the following classification

Group I Patients with uncomplicated hypertension No marked symptoms and no signs except elevation of blood pressure

Group II Patients with diastolic blood pressure of at least 110 mm Hg and marked symptoms such as headache dizziness and fatigue but without obvious signs of cardio-renal vascular damage

Group III Patients with signs of cardio-renal vascular disease

Group IV Patients with malignant hypertension

Table III *Treated series Jan 1st 1967*

Pats	Total	Dead	Causes of death			
			Cardio vascular	Renal	Cerebro vascular	Others
Men	2 5	56	31 (55)	7 (13)	13 (23)	5 (9)
Women	208	29	8 (28)	5 (17)	15 (5?)	1 (3)

2 *Treated series*

Up to 1955 we had undertaken pilot studies with the drugs which were then available for reduction of blood pressure The ganglion blocking drugs caused, on the whole such serious side effects that they could not be used except for specially selected cases

In January 1955 we started systematic treatment of hypertensive patients At first we used principally Rauwolfia combined with hydralazine and from 1957 Rauwolfia combined with thiazides From 1960 Rauwolfia has also been combined with guanethidine in the more serious cases in less severe cases a combination of α methyl dopa and thiazides has been the most usual line of treatment In a few cases a single dru e g a thiazide has been sufficient to give adequate control of the hypertension

The present investigation therefore does not give the results of treatment with any individual drug but rather the results of the most adequate treatment which we could achieve It is not possible to be certain that patients have followed our advice in every detail but we have the impression that they have done so

There were initially 553 patients (285 men and 268 women) However 120 patients (60 men and 60 women) have not been seen by us since the first year some because they did not come for control as arranged and others who as planned continued to be controlled by the r local doctor or at another hospital There remain 433 patients (?25 men and 208 women) (Table II) whom we in co-operation with other doctors have treated for more than one year These patients have provided the basis for our calculations of death rates We have in these calculations used whole years of observation and observations have been brought up to January 1967 A few patients who stopped treatment have s nce died patients who died during the year in which treatment ceased are included as dead

Four patients died during the first year of treatment A 40-year-old woman died of metastasing mammary carcinoma she had in addition a malignant hypertension that was quite intractable A 60 year-old woman died after 11 months of treatment of hypertension which it was not possible to get under control death was due to cerebral haemorrhage One man died of cerebral haemorrhage after three months of treatment at the beginning of the treatment he already had a cerebral thrombosis Another man died suddenly in the street after one month of treatment possibly from myocard al infarction

The average age of the patients in the treated ser es at the t me of starting treatment was 50 years By the WHO system of classification 109 men and 108 women belonged to Group I (uncomplicated hypertension) 55 men and 60 women to Group II (with cardiovascular complications) and 61 men and 40 women to Group III (with other complications) (Table II) The Group I patients had the lowest average a_e and the Group III patients the highest

As the untreated sample was examined and classified according to Hammarstrom and Bechgaard s system the treated sample has also been classified and evaluated by the same method (Table II) Only ten men and ten women belonged to Group I and only five men and three women had malignant hypertension (Group IV) The other patients were divided between Groups II and III

RESULTS

1 *Untreated series*

At the review in January 1967 we found that of the original 290 patients 175 had died (71 men and 104 women) For these the causes of death (Table I) were cerebrovascular 43 for men and 51 % for women cardiovascular 27 for men and 19 / for women renal 6% for men and 15 ° for women The remaining 24 o of the men and 15 ° of the women died of causes unrelated to hypertension The relative importance of these unrelated causes has increased during the last few years

Among those dying of cardiovascular causes were nine men and seven women who died of myocardial infarction This shows a relatively low rate of coronary deaths particularly among women

2 *Treated series*

By January 1967 of the original 433 patients 85 (56 men 29 women) had died For these the causes of death (Table III) were cerebrovascular 23 ° for men and 52 for women cardiovascular 55 ° for men and 28 for women renal 13 for men and 17 for women causes unrelated to hypertension 9 for men and 3 o for women

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IRON STORES IN ALCOHOL ABUSERS

I Liver Iron

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Abst act Storage iron has been examined in aspiration liver biopsy specimens obtained from 41 alcohol abusers without cirrhosis of the liver and from 20 hematologically normal males with gall bladder disease who were other wise healthy No one had a history of hemorrhage or iron medication. Only male subjects were examined The alcohol cs consumed mainly distilled alcoholic beverages Histochemical iron was estimated in parenchymal cells and in Kupffer cells and graded 0 to 4+ Stainable iron in parenchymal cells of grade 1+ or more was present in 15 of the 20 controls (75 %) and in 25 of the 41 alcohol abusers (61%) In Kupffer cells stainable iron was present in three of the controls (15) and in 15 of the alcohol abusers (37%) Two alcohol abusers had stainable iron in the Kupffer cells but not in the paren chymal cells Thus stainable iron of grade 1+ or more in either parenchymal cells or Kupffer cells was present in 27 of the 41 alcoholics (67%)

In 37 alcoholics and in 20 controls non hemin iron in the liver was determined chemically The mean non hemin iron concentration related to dry weight was significantly lower in the alcohol abusers (68 3 $\pm$ 5 8 mg per 100 g dry weight) as compared with the controls (104 4 $\pm$ 11 2 mg) With protein as reference the mean iron concentration in the liver was lower in the alcohol abusers (148 4 $\pm$ 11.5 mg/100 g protein) as compared with the con rols (185 7 $\pm$ 20 6) The d fference was however not signifi cant No a cohol c had a non hemin iron concentration above the range of the controls Thus the present study d d not reveal increased iron stores in alcohol abusers The results rather indicated diminished iron stores in alcohol abusers as compared with controls

Though conclusive evidence is lacking it is often believed that chronic abuse of alcohol and in creased iron stores are associated (20 40 43 44 49 50) Neverth less some circumstances and some investigations lend support to such an as sociation Sheldon (42) suggested that hemo-chomatosis is due to an inborn error of metabo-lism and pointed out the high frequency of alco holism in this disease MacDonald (24-28) con sidered hemochromatosis an acquired disease and suggested that conditions associated with alco holism are important causes of the disease The high iron content of some alcoholic drinks (1 26 35) may contribute to increased iron stores Alco-hol has also been reported to increase the absorp tion of ferric iron (7) Steatosis common in alco holics was reported to be associated with in creased iron absorption in man (12) and experi mental steatosis was associated with increased radio iron absorption in the rat (34) Increased absorption of radio iron has been reported in cirrhosis of the liver (6 10 11 16 50) and in chronic pancreatitis (8 9) The latter was not however confirmed by Balcerzak et al (2) Re ports on the effect of chronic alcohol abuse on the size of the iron stores in subjects without cirrhosis of the liver are few and their results difficult to evaluate Most studies have be n made on autopsy material by histochemical methods Acceptable control series have been lacking Other factors influencing the iron stores such as sex blood loss blood transfusions iron administration gastric resection or anemia not due to iron de ficiency have not been adequately taken into ac count

The present investigation was designed to study the effect of long standing alcohol abuse on the size of the liver iron stores in subjects without cirrhosis of the liver Individuals with a history of blood loss blood transfusions or iron medica tion were not included The content of non hemin iron in the liver was determined in aspiration biopsy specimens by quantitative chemical analysis

and by histochemical estimation. Results from alcohol abusers were compared with those from controls. The aspiration biopsy specimens from the controls were obtained at operation for uncomplicated gall bladder disease. Only males were studied.

METHODS

Liver biopsy specimens were obtained by an aspiration technique. Needles with an internal diameter of 1.6 mm were used. In the controls the liver biopsy specimen was taken from the margin of the right liver lobe at the start of operation; in the alcoholics percutaneous transthoracic biopsy was performed. Care was taken to avoid contaminations with iron from the environment. Acid-washed utensils, iron-free water and reagents of analytical grade were used. A piece of the biopsy specimen (about 0.5 cm long) was fixed in 10% neutral formaldehyde for 24 hours, embedded in paraffin and cut into 3 μ sections which were stained with hematoxylin and eosin. The staining for hemosiderin was done with potassium ferrocyanide according to the modification of Hutchison (17). To assess the amount of hemosiderin in liver biopsy specimens the sections were examined at ×600 magnification.

For parenchymal liver cells the following grading was used: Grade 0. No stainable iron or only obviously extracellular artefacts. Trace. Isolated fine granules in the whole preparation, difficult to determine whether artefacts or not. Grade 1+. Definite fine granular hemosiderin in single cells or in small scattered cell groups. Grade [illegible]. Fine granular hemosiderin in a few or several cells present in most lobules. Grade 3+. Mainly fine granular hemosiderin but also large granules present in the periphery of all lobules. Grade 4+. Very abundant amount of stainable iron in the major part of the lobule which to a great degree consists of gross granules and aggregations of hemosiderin.

For Kupffer cells the following grading was used: *Grade 0. No obvious histiocytic iron. Trace. Iron in* single Kupffer cells, difficult to determine whether artefacts or not. Grade 1+. Definite intracellular hemosiderin in single Kupffer cells. Grade 2+. Moderate amount of histiocytic iron (hemosiderin) in one or several Kupffer cells in all fields. Grade 3+. Hemosiderin in most identifiable Kupffer cells. The iron consists to a great degree of gross aggregations and the cells are swollen. Grade 4+. Hemosiderin in all identifiable Kupffer cells which are present in abundant amounts. The iron consists mainly of gross hemosiderin aggregations and the cells are mostly greatly enlarged.

The rest of the biopsy specimen (mean dry weight of about 10 mg) was transferred into a small weighing vessel and the dry weight determined after freeze drying to constant weight. The specimen was then homogenized in an all-glass homogenizer. The homogenate was transferred into a graduated tube and made up to [illegible] ml. Duplicate [illegible] ml aliquots of the homogenate were taken for analysis of total non-hemin iron after extraction with 2.8 N hydrochloric acid as previously described (46). To render analysis of the small specimen reliable the extracts were diluted to 10 ml instead of 25 ml. Proportionately less of the reagents were used. The standard deviation of a single determination calculated from 44 duplicate determinations with a mean iron content of 2.79 μg was 0.107 μg, corresponding to a coefficient of variation of 3.8%.

In addition to dry weight, protein was used as a reference for the non-hemin iron determination. Alkali-soluble protein was determined as follows: an aliquot of 0.5 ml of the liver homogenate was extracted with 0.5 ml of 0.[illegible] N NaOH for about 18 hours at room temperature. During the first hours the sample was stirred regularly with a glass rod. The sample was then centrifuged and 0.1 ml of the supernatant (containing approximately [illegible]0 μg of protein) was taken for protein analysis according to Lowry et al. (22) as modified by Rieder (38). Solutions of human serum albumin (Kabi) were used as standards and treated in the same way as the homogenate. The error of the method calculated from 43 duplicate determinations with a mean protein content of 48.6 μg was 0.87 μg, corresponding to a coefficient of variation of 1.8%. All determinations were performed in duplicate.

The determination of non-hemin iron in small aspiration specimens according to the present procedure was compared to the analysis of large wedge specimens according to the method previously described (46). From 11 cadaver livers 32 aspiration specimens and 3[illegible] wedge specimens taken from the same sites were analysed by these procedures. The mean iron concentration (with protein as reference) in the aspiration specimens was 180.3±31.5 mg per 100 g protein and the corresponding value in the wedge specimens was 161.0±33.0 mg per 100 g protein. There was a marked correlation between the results of the two procedures ($r = 0.98$). The equation of linear regression of the iron concentration in the aspiration specimens upon the iron concentration in the corresponding wedge specimens was $y = 0.94x + [illegible]9$. The difference between the means (19.3±5.8) was, however, significant ($t = 3.3$, $p < 0.01$). The mean iron content ([illegible]0 μg) in the wedge liver specimens was approximately seven times the amount present (3 μg) in the aspiration liver specimens.

The total error of the aspiration technique, including the variation of the iron content in different parts of the liver, was determined as follows. Two aspiration liver biopsy specimens were taken at a distance of 5–10 cm from each other in [illegible]1 subjects at operation for gall bladder disease. The standard deviation of a single biopsy specimen was 7.6 mg per 100 g dry weight (mean iron concentration was 97 mg per 100 g), corresponding to a coefficient of variation of 7.8%. With protein as reference the total error of the aspiration technique was [illegible]0.9 mg per 100 g (mean iron concentration was 184 mg per 100 g), corresponding to a coefficient of variation of 11.3.

Quantification of visible fat in liver biopsy specimens was done with a Zeiss Integration Eyepiece I (3). One thousand points (40 positions of the grid for each slide) per slide were counted. The standard deviation of a single determination calculated from 10 duplicate determinations

Table I *Concentration of liver iron alkali soluble liver protein and visible liver fat in 20 control subjects*

Subject	Age	Hb (g/100 ml)	Serum iron (μg/100 ml)	TIBC (μg/100 ml)	Liver biopsy specimen: Chemical analysis: Fe dry wt (mg/100 g)	Fe/protein (mg/100 g)	Prot /dry wt (g 100 g)	Histochemical estimation: Visible fat ( )	Stainable iron (grade 0–4+): In parenchymal cells	In Kupffer cells
A A	67	13 6	177	345	103	172	60 0	0 8	+	0
A R	19	15 1	79	400	45	86	52 1	0 1	0	0
C R	44	15 0	124	360	74	132	56 1	0 0	+	0
E S	31	14 6			130	197	66 1	0 0	+	0
H S	28	15 4	192		92	172	53 4	1 9	+ +	0
J An	81	14 1	145	378	105	203	51 9	0 5	+	0
J Å	47	14 3	171	366	104	184	56 7	0 0	+	0
J V	30	13 5	81	314	153	258	59 1	0 0	+ +	0
L A	4	15 3	203	337	247	485	51 0	0 0	+ + +	+ +
L. K E.	33	13 5			45	85	52 8	0 0	0	0
M M	61	13 6	59	261	113	177	63 9	0 1	+ +	0
N V	72	13 8	127	296	75	152	49 6	0 0	+	0
N H	63	14 0	137	350	61	125	49 2	0 1	Trace	0
N P	65	14 1	95	281	61	111	54 8	0 0	0	0
O A	26	15 5	181	314	108	193	56 1	0 0	+ +	+
P B	18	13 5	99		62	111	56 1[a]	0 0	0	0
P A	40	14 5	151	400	143	233	61 4	2 4	+ +	0
S D	21	13 6	138	339	99	173	57 2	0 2	+	0
T R	35	14 8	1 6	397	192	333	57 7	0 5	+ + +	+
L. A	66	15 5	170	332	75	13[illegible]	56 7	2 0	+	0

[a] Pred cted values

with a mean value of 7 4 was 0 7% corresponding to a coefficient of variation of 10

Serum iron was determined according to the method of Laurell as described by We nfeld (46) and total iron binding capacity according to Peters et al (36) Hemoglobin was determined photometrically as cyanmethemoglobin.

Serum bilirubin and alkal ne phosphatase were deter mined according to Roos (39) thymol turbidity acco d ing to MacLagan (29) and the serum glutam c-oxalaceti transaminase (SGOT) by a modification of the method of Karmen *et al* (18)

The iron content of d fferent wines commonly used by alcoholics in Goteborg was analysed by a wet ash n method (23)

MATERIAL

No subject included in the study had a history of blood loss, had undergone gastric resection or had received blood transfusions or iron med cation

Controls (Table I)

Twenty males with a mean age of 44 years (range 19–81 years) were stud ed All were admitted for cholecystectomy but were otherw se healthy and in a good nutr tional state and they were hematologically normal The r lower limit for hemoglobin concentration wa 13 5 g per 100 ml

Gall bladder stone was found in all but one (H S) who had gall bladder cholesterosis. Only a few had a hi tory of acute cholecystitis. None had clin cal symptoms of acute cholecystitis or fever at the time of operation Liver function tests were normal in all but two (H S and J A) who had sl ghtly increased glutamic-oxalaceti transaminase (SGOT) and one who had slightly increased *thymol turbidity (N V) None had a h story of* jaundice but one (L A) had a slightly increased serum bilirubin concentration In this patient microscopic examinat on of the liver biopsy specimen revealed a few small bile thrombi Otherwise the microscopic examination showed no changes apart from slight steatosis in some pat ents No control subject had more than 2.5% of visible fat

There was no abuse of alcohol. Three were total abstainers, ten consumed insignificant amounts, five con sumed half a litre of distilled alcoholic beverages or less per month one (H. S) consumed one bottle of whisky and two bottles of red wine per month and one (T R.) consumed two bottles of red wine per month and half a litre of whisky per month Beer consumption was low in all

Table II *Duration of alcohol abuse wine consumption concentration of liver iron alkali soluble liver protein and visible liver fat in 41 alcoholics*

			Excessive wine consumption					Liver biopsy specimen					
											Chemical analysis		
Pat	Age	Duration of alcoholism (y)	Bottles/ week	Duration (y)	Hb (g)	Serum iron (μg)	TIBC (μg)	Visible fat (%)	Stainable iron P^a	K^b	Fe/dry wt (mg/ 100 g)	Fe/prot (mg/ 100 g)	Prot/dry wt (g/100 g)
K G K	47	15			13 4	103	307	2 6	0	0			
Ah G	58	30			12 4	84	339	0 2	+	0			
S B	34	15			14 4	10.	317	0 0	+ + +	+			
An O	55	5			14 2			32 3	+ +	+ + +			
E E	55	.0			12 8	153	441	5 3	+ + +	+ +			
K M	47	10			13 5	139	362	0 3	+	+	170	234	31 2
An G	59	15			11 0	145	380	1 5	+	+ +	63	125	50 7
B O	49	10			12 3	112	332	1 8	+	0	55	97	36 4
B N	55	10	2	5	13 8	63	375	0 8	+ +	0	88	169	52 0
C G	50	15			11 7	94	276	6 7	Trace	0	71	213	33 ?
F S	41	10			14 6	300	360	4 7	+	0	72	187	38 7
F L G	35	5			1? 4			12 7	+ +	0	77	130	39 ?
H Sg	30	10			12 9	142	409	0 8	+	0	48	107	44 2
J L	35	10			15 2	269	328	6 7	+	0	64	168	38 0
L Go	50	25	3	5	12 9	156	406	7 1	+ +	+ +	70	164	42 6
L N	54	20	3	5	12 5	113		0 0	0	0	23	48	47 6
M E	58	35	3	5	12 1			0 6	0	0	67	117	37 ?
M L	46	10			12 3	100	223	6 4	0	0	73	156	47 0
O H	56	15			12 5	132	240	9 1	+	+ + +	80	241	33 1
S H	4?	15			14 3	213	326	11 5	+	+	35	118	29 4
An L	24	5			12 4	150	390	0 1	0	0	81	128	63 5
K C	55	20			15 7	189	390	18 4	0	0	34	96	35 5
T L	43	5			15 0	2 0	461	0 8	0	+	61	117	32 1
V H	48	10	7	3	14 3			0 8	0	0	65	126	31 6
Å H	70	30			12 5	86	299	0 0	0	0	39	87	45 2
B Ae	52	?0			11 6	68	4 0	12 5	+	0	56	136	41 1
D R	46	15	5	10	14 8	196	414	22 8	0	0	27	77	34 7
J A	37	5			13 0	?6	470	30 8	0	0	17	70	24 7
W T	6.	0	2	5	12 3	1?8	295	6 8	+	+	81	142	56 9
M K	74	10			11 9	63	383	5 1	Trace	− +	70	179	39 0
M	63	20	7	7	15 0	91	273	2 9	+ − +	+	219	412	53 2
S P	49	10	3	5	13 9	115	281	17 4	+ +	+	105	287	36 7
G S	57	10			13 2	117	98	. 6	0		47	128	36 8
S E	63	10			13 2	173	360	3 5	+	0	77	158	48 9
T H	64	10			11 1	23	320	0 9	+ −	0	37	100	37 0
K J	60	25	7	5	12 1	73	3.6	8 9	+	0	63	138	45 8
W O	49	10			16 1	161	296	12 0	0	0	49	95	51 7
B G	36	5			11 8	77	374	0 0	+	0	79	148	53 4
L Gr	44	5	5	5	15 4	94	355	0 0	+ +	0	99	198	49 9
S S	58	?0			13 6			14 2	Trace	0	28	66	42.0
W F	33	5			14 3	141	365	0 8	+	+	98	181	54 0

[a] Parenchymal liver cells [b] Kupffer cells

Alcohol abusers (Table II)

Forty-one male alcohol abusers with a mean a e of 50 years (range 24–70 years) were studied Only patients with an alcohol intake converted to 40 ethyl alcohol of at least 1 /₄ l per week for at least five years were included Most of the patients were alcoholics in the medical and social sense and all but 11 had been admitted previously to mental hospitals psychiatric wards or alcoholic institutions

At the time of investigation 23 of the patients were in a mental hospital (Clinic IV Lillhagen s Hospital) for chronic and acute alcoholism and the rest in a somatic ward (Department of Medicine I Sahlgren s Hospital) The cause of admission for the latter was in most cases alcoholic sequelae such as polyneuritis epileptic seizures, cardiomyopathy and liver steatosis

Liver biopsy was performed only in patients who had increased SGOT values Patients with hemoglobin concentrations below 11 g per 100 ml were not included.

Exact information as to the quantity of alcohol con

sumed, length of severe alcohol abuse and dietary habits could not be obtained from these for the most part, in tellectually impaired patients The figures for the duration of severe alcohol abuse in Table II are minimum values and probably underestimated in most cases

The consumption of distilled alcoholic beverages ex ceeded one litre per week in nearly all patients and averaged seven litres per month. Wine consumption of one bottle per week was common but only 11 had con sumed two or more bottles of wine per week for more than a year (Table II) These con umed d stilled alcohol c beverages as well. Beer consumption wa moderate in most patients.

Deficient and irregular dietary habits were common and most had lengthy periods during which alcoholic bevera es constituted the main caloric source

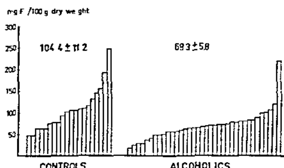


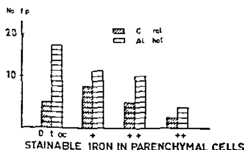
Fig 2 Distribution of the chemical liver iron concentra tion related to dry weight

RESULTS

Histochemical Iron

Controls (*Table I Fig 1*)

In *parenchymal liver cells* stainable iron of grade 1+ or more was present in 15 of the 20 controls (75) Eight had stainable iron of grade 1+ five had grade 2+ and two had grade 3+ In *Kupffer cells* stainable iron was visible in three subjects of these the histochemical iron was graded 1+ in two and 2+ in one All who had stainable iron in Kupffer cells had also visible iron in parenchymal liver cells



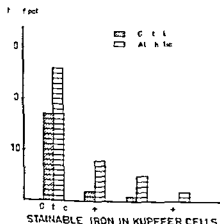


Fig 1 Distribution of the controls and alcoholics as to the grade of histochemically demonstrable iron.

Alcohol abusers (*Table II Fig 1*)

In *parenchymal liver cells* stainable iron of grade 1+ or more was present in 25 of 41 subjects (61°) Eleven had grade 1+ 10 had grade 2+ and four had grade 3+ In *Kupffer cells* stainable iron was present in 15 cases Eight had grade 1+ five had grade 2+ and two had grade 3+ Two had stainable iron in Kupffer cells but not in parenchymal liver cells Thus stainable iron in either parenchymal liver cells or Kupffer cells was present in 27 of the 41 alcohol abusers (67) Though stainable iron in the liver was slightly more common in the controls the difference was not statistically significant

Chemical Determination of Non hemin Iron Dry Weight as Reference

Controls (*Table I Fig 2*)

The mean non hemin iron concentration per 100 g dry weight was 104 4 ± 11 2 mg with a range of 45 to 247 mg

Alcohol abusers (*Table II Fig 2*)

The mean non hemin iron concentration of the alcohol abusers was 68 3 ± 5 8 mg per 100 g dry weight with a range of 17 to 219 mg The mean of the alcohol abusers was significantly lower ($p < 0\ 01$) than that of the controls

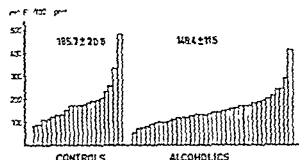


Fig 3 Distribution of the chemical liver iron concentration related to protein.

Protein as Reference

Controls (Table I Fig 3)

The mean non hemin iron concentration per 100 g protein was 185 7 ± 20 6 with a range of 85 to 485 mg

Alcohol abusers (Table II Fig 3)

The mean non hemin iron concentration was 148 4 ± 11 5 with a range of 48 to 412 mg per 100 g protein The mean of the alcohol abusers was lower than that of the controls but not significantly so (0 10 > p > 0 05)

The mean iron concentration (170 7 ± 30 6 mg per 100 g protein) of those who had consumed at least two bottles of wine per week for more than one year (on an average five years) was not significantly different from the mean (138 6 ± 9 5) of those who consumed smaller quantities of wine

There was no relationship between the duration of severe alcohol abuse and liver iron concentration (r = 0 07) The mean liver non hemin iron concentration of those with hemoglobin values of 13 0 g per 100 ml or more (160 4 ± 20 3 mg per 100 g protein) was not significantly different (p > 0 30) from the mean of those abusers who had hemoglobin levels below 13 0 g (136 4 ± 10 6 mg per 100 g protein)

There was no significant correlation of the regression of the liver iron concentration with protein as reference upon visible liver fat (r = 0 16 p > 0 10)

Relation Between Iron Estimated Histochemically and Non hemin Iron Determined Chemically (Figs 4 and 5)

In the *controls* with dry weight as reference the difference was significant (p < 0 005) between the mean iron concentration of those who had no stainable iron in parenchymal cells (53 3 ± 4 8) and those who had stainable iron of grade 1+ (95 6 ± 7 0) The mean of those with grade 2+ (121 8 ± 11 4) was considerably higher than that of those with grade 1+ but not significantly so (0 10 > p > 0 05) With protein as reference there was approximately the same degree of overlapping

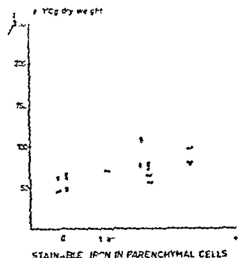


Fig 4 Relationship between histochemically stainable iron and chemical liver iron concentration with dry weight as reference base Open circles represent controls, filled circles represent alcoholics.

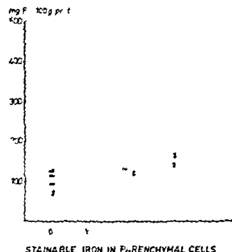


Fig 5 Relationship between histochemically stainable iron and chemical liver iron concentration with protein as reference base Open circles represent controls, filled circles represent alcoholics.

In the *alcoholics* with dry weight as reference there was a very high degree of overlapping there was no significant difference ($0.10 > p > 0.05$) between the means of those without histochemical iron in the liver (47.5 ± 7.2) and those with grade 1+ in parenchymal cells (66.9 ± 7.3) and the absolute difference between the mean of those with grade 1+ (66.9 ± 7.3) and grade 2+ (79.2 ± 4.8) was small. With protein as reference there was a significant difference ($p < 0.01$) between the mean of those alcoholics without histochemical iron in the liver (100.0 ± 10.1) and those with grade 1+ in parenchymal cells (147.8 ± 13.0). The mean non-hemin iron values of the same histochemical gradings in controls and alcoholics were in better agreement when protein was used as reference (compare Figs. 4 and 5).

Hemoglobin, Serum Iron and Serum Iron binding Capacity

The mean hemoglobin concentration of the alcoholics (13.3 ± 0.2 g per 100 ml) was significantly lower than that of the controls (14.4 ± 0.2) and ranged from 11.0 to 16.1 g per 100 ml.

The mean serum iron concentration of the alcoholics (128 ± 10 μg per 100 ml) was not statistically different from that of controls (136 ± 10). Two alcohol abusers (J. A. and T. H.) had serum iron values below 60 μg per 100 ml (26 and 23 μg per 100 ml respectively).

The mean TIBC level of alcohol abusers (348 ± 10 μg per 100 ml) was not different from that of controls (342 ± 10 μg per 100 ml).

Visible Fat

No visible fat was present in 10 of the 20 controls. In the others the mean value was 0.9 % with a range of 0.1 to 2.4 %.

In the alcohol abusers visible fat was present in 36 of the 41 patients examined. The mean value of these was 7.6 % with a range of 0.1 to 32.3.

Protein Concentration

In the controls the mean protein concentration was 56.1 ± 1.2 g per 100 g dry weight ($n = 17$). In the alcohol abusers the mean protein concentration was 46.0 ± 1.6 g per 100 g dry weight and was significantly lower ($p < 0.001$) than that of the controls.

Table III. *Iron concentration in cheap wines commonly used by alcoholics in Goteborg*

Wine	No. of brands tested	Iron concentration (mg/l) Mean	Range
Red	6	11.0	7.6–14.3
White	6	8.6	6.0–14.6

Relation Between Protein and Visible Fat

In the controls who had only small amounts of visible fat (less than 2.5 %) there was no correlation between protein concentration and visible fat: the mean protein concentration of those who had visible fat (56.4 ± 1.5 g per 100 g dry weight) was not significantly different from those who had no visible fat (55.7 ± 2.1).

In the alcoholics, many of whom had considerable steatosis, there was a negative correlation between the protein concentration and the amount of visible fat. The correlation coefficient was -0.64 ($p < 0.001$) and the equation of linear regression of protein concentration upon the amount of visible fat was $y = 51.2 - 0.81x$.

Iron Content of Wine

The iron content of 12 different cheap wines commonly used by alcoholics in Göteborg was analysed by a wet ashing method. The mean iron concentration was about 10 mg per litre (Table III).

DISCUSSION

The mean non-hemin iron concentration of the controls in the present study is similar to those given in the literature from different parts of the world (Table IV) (31, 32, 33, 41).

In a previous male control series (47) a mean value of 80.2 ± 9.6 mg per 100 g dry weight was obtained on wedge liver specimens. The higher mean of the present investigation (104.4 ± 11.2) is probably due to the somewhat higher values obtained when analysis is performed on small aspiration specimens. Slight iron contamination is inevitable. This should not influence samples with a large iron content (wedge biopsy technique) but might do so in samples with low iron content

Table IV *Reported normal values for liver non hemin iron concentration in previously healthy subjects killed in accidents or suicides*

Authors	Year	Country	No of subjects studied		Liver non hemin iron concentration (mg 100 g dry weight)		Cause of death
			Males	Females	Mean	S E of mean	
Schairer et al	1948	East Germany	18		95 0[a]	12 7	Accident
Meier et al	1959	East Germany	136		95 4		Accident or suicide
Morgan et al	1963	Australia	18	3	81 5	10 7	Accident
Mayet et al	1964	South Africa	85		89		Accident

[a] Recalculated from wet weight to dry weight assuming a liver water content of 70 per cent

(aspiration biopsy technique) The high degree of reproducibility obtained in liver specimens taken from the same liver indicates that the iron contamination was of the same magnitude If the present results are corrected for the difference between the two methods the mean for the controls of the present investigation should be reduced to 80 mg per 100 g dry weight

The results have shown that the mean iron concentration of the liver related to dry weight was significantly lower in the alcohol abusers as compared with the controls Iron concentration with weight as reference would give reliable information as to changes of the total iron content of the liver provided the weight and size of the liver remains constant Changes in liver size by dilution with lipids or glycogen may introduce difficulties in interpretation In the present study many alcoholics had severe steatosis and increased liver size This would imply a decrease in the iron concentration if wet weight is used as reference The decrease in iron concentration would be still greater if dry weight were used as reference since fat has a low water content If it is assumed that the total amount of liver protein is unaltered with different degrees of steatosis then the iron concentration with protein as reference would give more reliable information on changes in the total liver iron content This is supported by the fact that the non hemin iron values in alcoholics and controls with the same histochemical grading were more in agreement when protein was used as reference (compare Figs 4 and 5)

In a study of Martinsson et al (30) there was a negative correlation between liver glycogen concentration and protein in various experimental conditions resulting in different glycogen concentrations The relation of several substances in the liver to liver protein was however unchanged, indicating that protein may be used as reference substance In our study small amounts of visible fat did not significantly influence the protein concentration but with larger amounts of fat the protein concentration on a dry weight basis decreased When relating iron to protein the mean iron concentration was lower in the alcoholic group as compared with the control group but the difference was not significant It is, however possible that total liver protein is reduced in severe steatosis In such cases iron related to protein as a parameter of total liver iron should give too high values Thus liver non hemin iron stores of the alcoholic group were definitely not increased but might be decreased as compared with the control group Nor did the estimation of stainable iron in parenchymal liver cells indicate increased liver iron stores in alcoholics histochemically visible iron in parenchymal cells was slightly more common in controls The more frequent finding of stainable iron in the Kupffer cells of alcoholics did not reflect increased non hemin liver iron stores but might be the result of liver cell injury and hyperactivity of the reticuloendothelial system.

The mean hemoglobin concentration of the alcoholics was significantly lower than that of the controls In the alcoholics with anemia (hemoglobin less than 13 0 g per 100 ml) liver iron concentration did not indicate iron deficiency and only in five of twenty alcoholics with anemia was no stainable iron found in the liver This implies that lack of iron was not the limiting factor for their hemoglobin synthesis Other causes of anemia

in alcoholics have recently been discussed (43 45) It is to be expected that if the anemia in the alcoholics had been corrected before liver biopsy even lower liver iron concentrations would have been found in this group

Although it is a common view that liver iron stores often are increased in subjects with heavy alcohol consumption reliable studies supporting this view are lacking except for the studies of iron stores in the Bantu (4 5 14 15 31) Thus Mayet and Bothwell (31) who determined liver non hemin iron chemically in subjects dying an acute traumat ic death found that the median liver storage iron concentration of a Bantu group was more than three times that of a male white group The iron over load in the Bantu is thought to be due to the high dietary content of iron most of which is pres ent in alcoholic drinks (50–100 mg daily) (5) Alcoholic drinks in other countries may also con tain much iron According to Amerine (1) the mean iron content of wines in France is 8 8 mg per litre and in Italy 16 0 mg per litre In a recent study by Perman (35) the iron content of different red wines obtained from provincial inns in Northern Italy was 33 mg per litre As wine alcoholics usually consume several bottles of wine per day their iron intake may be many times the normal Lereboullet et al (21) in France studied the presence of stainable iron in liver biopsy spe cimens obtained at autopsy or by percutaneous biopsy in 92 cases of chronic alcoholism without cirrhosis of the liver and compared the results with those obtained in 42 non alcoholics with normal liver histology They found stainable iron in 59 per cent of the alcoholics and in 35 per cent of the non alcoholics In Italy Dominici et al (13) found stainable iron in 41 out of 68 cases of chronic alcoholism There was no information as to the frequency of stainable iron in normals Ditt rich (12) in Austria found evident siderosis in 21 per cent of 300 unselected biopsy specimens and in 69 per cent of alcoholics with steatosis Besides countries with great wine consumption Zimmerman et al (51) in USA found stainable iron in only two out of 15 alcoholics without hepatic disease MacDonald et al (27) in the same country found stainable liver iron in 53–80 of unselected autopsy materials

For the evaluation of the normal range of liver iron all factors which might alter the normal iron balance must be considered Hemorrhage at any time before the study might lower the iron stores since once reduced they are rebuilt very slowly In comparing iron stores in different groups a distinction must be made between men non men struating women and menstruating women (46) Thus an appreciable proportion of women (espe cially menstruating ones) would lower the mean liver iron value of the group Consequently the comparison of such a control group with an alco holic one composed mainly of males would give a false impression of increased iron stores in the latter In some of the above cited studies no con trol material for comparison was presented and in those in which it was relevant data of its composition were lacking It should also be pointed out that a material of unselected liver biopsy specimens cannot be regarded as a control material Liver biopsies are usually performed on patients with gastrointestinal disturbances who are liable to have a negative iron balance Hence no conclusions can be drawn from the above studies whether iron stores are increased in alcoholics

In a recent study by Powell performed in Au stralia (37) liver specimens from subjects suffer ing sudden death were examined histochemically and chemically Alcohol consumption was as sessed by interviewing close relatives The material was divided into males and females and into those with heavy and light alcohol consumption Fourteen males with heavy alcohol consumption had significantly higher mean liver iron concentra tion than 33 males with light alcohol consump tion It was also stated that there was a significant correlation between alcohol consumption and hepatic iron and between iron ingestion from alcoholic beverages and hepatic iron Subjects who donated blood within 12 months of death were excluded However as mentioned above blood losses many years before a study may in fluence the size of the iron stores It is also our impression that subjects with cardiovascular disease who are on continuous anticoagulant therapy may have grossly decreased iron stores though a history of hemorrhage is lacking A large proportion of subjects in the study of Powell had cardiovascular disease but it is not stated if they were on anti coagulants Furthermore there was no information as to iron administration or blood transfusion As there was a significant correlation between iron ingestion from alcoholic beverages and hepatic iron it seems probable that the males with heavy

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IRON STORES IN ALCOHOL ABUSERS

II *As Measured with the Desferrioxamine Test*

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Abstract Desferrioxamine (DF) induced urinary iron ex cretion has been determined in 58 male alcohol abusers and in 6 hematologically normal males The mean DF induced iron excretion of the alcoholics was not different from that of the normal males In eight alcoholics how ever the iron excretion was above the range of the normals. In 13 controls admitted for non acute upper abdominal operations there was a statistically significant correlation between liver iron concentration and DF induced iron excretion but in 26 alcohol abusers no significant correlation was found The iron excretion in the alcoholics usually exceeded the value predicted from the liver iron concentration To elucidate this discrepancy the DF test was performed on admission and after an interval of 7-11 days in a group of alcoholics with a de clining SGOT level The DF induced iron excretion was lower at the time of the second test in 14 of 15 subjects The higher DF induced iron excretion at the first test was probably due to liver cell injury In five of the eight alcohol abusers with an iron excretion above the range of the controls liver biopsy was performed and did not show increased storage iron In two of the others there was a high SGOT level.

Thus increased DF induced iron excretion was present in some of the alcoholics but was probably due to in creased iron chelation caused by hepatic cell injury Alcoholics as a group did not have increased DF induced urinary iron excretion

Chronic alcoholism is often believed to be as sociated with increased iron stores (1 7 10 11 12 13) A previous study (6) however showed that the liver non hemin iron was not increased in subjects with longstanding abuse of distilled alcoholic beverages In the present study the iron stores were estimated in a larger group of alcohol abusers and in controls by means of the d s ferrioxamine (DF) test This test has been found valuable in the diagnosis of iron overload (8 14) and useful in evaluating iron stores in normal and iron deficient sub ects (2 3)

The DF induc d urinary iron excretion of alco holics was compared with that of controls and the relationship between the DF induced iron excretion and the non hemin iron concentration in liver biopsy specimens was studied Further more the influence of alcoholic liver injury on the DF test was investigated

MATERIAL

Only male subjects were included

Controls

Two control groups were studied No control subject had a history of hemorrhage and no one had undergone gastric resection They had never received blood transfusions or iron medication

Control group I (Table I)

In this group the DF induced iron excretion serum iron and iron binding capacity (TIBC) were determined. Twenty six healthy volunteers were stud ed The lower limit for their hemoglobin concentration was 13 5 g per 100 ml There was no abuse of alcohol f e were total abstainers ten consumed insignificant amounts four consumed half a litre of distilled alcoholic beverages per month and seven consumed about one litre per month Two of the latter consumed also two bottles of wine per month Beer consumption was low in all

Control group II (Table II)

The group comprised 13 males adm tted for upper ab dominal operations In this group the iron content of liver b opsy specimens as well as the DF induced urinary iron excretion were determ ned One was admitted for operation of a duodenal ulcer and the others for chole cystectomy They were otherwise healthy and in good nutritional state None had fe er or other clinical si ns of acute cholecystitis at the time of operat on The serum glutamic-oxalacetic transaminase (SGOT) le el was bor

Table I *Desferrioxamine (DF) induced urinary iron excretion in healthy male controls (Control group I)*

Subject	Age	Weight (kg)	Hb (g)	Serum iron (μg)	TIBC (μg)	DF induced urinary iron excretion (mg)	(μg/kg body weight)
A N	25	80	15 1	111	345	0 55	6 9
B S	22	65	13 7	225	331	0 70	10 8
L O	38	85	15 2	158	267	1 27	14 9
S K	26	75	14 6	114	296	0 72	9 6
V C	26	105	14 5	97	314	1 35	12 9
N S	33	67	14 6	141	375	0 51	7 6
L B	28	66	14 2	98	301	0 47	7 1
H Ö	57	70	13 6	72	271	0 59	8 4
P G	57	89	16 4	149	405	0 88	9 9
A K	43	80	13 9	102	409	0 55	6 9
L L	21	78	13 9	148	311	0 82	10 5
L D	42	75	14 1	189	348	0 76	10 1
O S	33	78	13 8	136	340	0 74	9 5
G G	51	70	15 7	14[illegible]	377	0 86	12 3
O L	23	78	15 0	96	322	0 67	8 6
S B	53	78	14 8	124	366	0 90	11 5
M J	24	71	15 0	118	278	0 69	9 7
M T	60	83	15 8	123	351	1 04	12 5
E N	37	82	14 8	86	348	1 14	13 0
S E	51	76	14 6	92	336	0 63	8 3
A P	22	58	14 5	126	289	0 68	11 7
K K	34	74	13 6	208	264	0 73	9 9
G B	22	73	14 1	161	417	0 66	9 0
L S	55	90	15 6	113	290	0 90	10 0
K A	58	72	13 5	181	307	0 75	10 4
S H	39	80	14 3	105	336	0 74	9 3

derline in two (H S and J An) but normal in the rest of the patients The serum bilirubin and thymol turbidity test were normal in all. Microscopic examination of the liver biopsy specimens revealed no changes apart from slight steatosi in som patients Only one consumed more than one litre of distilled al oholic beverages per month five ere total abstainers three consumed insignificant amounts and four consumed from half a litre to one bottle per month Two of the latter subjects (H S and T R) also consumed two bottles of wine per month One patient (E. G) consumed half a litre to one bottle of whisky per week He had however abstained from alcohol consumption during the last few weeks before admission and had normal liver function tests He had no significant

Table II *DF induced iron excretion and liver non hemin iron in 13 male patients admitted for upper abdominal operation (Control group II)*

Pat	Age	Weight (kg)	Hb (g)	Serum iron (μg)	TIBC (μg)	DF induced urinary iron excretion (mg)	(μg/kg body wt)	Liver non hemin iron (mg/100 g protein)
H S	28	80	15 4	192		1 16	14 5	172
J V	30	61	13 5	81	314	0 58	9 5	258
N R	44	86	14 6	160	366	1 12	13 0	332
S D	21	67	13 6	138	339	0 57	8 5	173
T R	35	64	14 8	176	357	0 97	15 2	333
J An	81	82	13 9	36	365	0 59	7 2	183
J A	49	73	14 1	145	378	0 74	10 1	203
N H	63	75	14 0	137	350	0 61	8 1	125
N P	65	60	14 1	95	281	0 49	8 2	111
P A	40	86	14 5	151	400	1 24	14 4	233
A A	67	82	13 6	177	345	0 76	9 3	172
A R	19	99	15 1	79	400	0 69	7 0	86
E G	50	92	14 8	149	304	0 95	10 3	207

Table III *DF induced iron excretion and liver non hemin iron in alcoholics (Alcohol abusers group I)*
Figures in brackets represent analysis of blood samples obtained at a different time than the DF test

Pat	Age	Weight (kg)	Duration of al ohol abuse (y)	Excessive wine consumption		Hb (g)	Serum iron (µg)	TIBC (µg)	DF induced iron excretion		SGOT (units)	Liver non hemin iron (mg/100 g protein)
				Bottles/ week	Duration (y)				(mg)	(µg/kg body weight)		
Ah G	58	67	30			12 4	84	339	0 45	6 7	25	
An G	59	77	15			11 0	145	380	0 74	9 6	45	125
An L.	4	63	5			12 4	(150)	(390)	0 49	7 8	37	123
Ba G	40	96	5			14 1	98	415	0 55	5 7	50	
B O	49	60	10			12 3	112	332	0 72	12 0	35	97
B A	51	97	5			14 6	105	310	1 0	10 5	43	
B N	55	62	10	2	5	13 8	(63)	(375)	0 78	12 6	55	169
Bl Ö	47	70	5			14 0	118	548	0 62	8 9	50	
B J	27	65	5			14 0	55	293	0 32	4 9	75	
C G	50	61	15			11 7	94	276	0 84	13 8	55	213
D R	46	74	15	5	10	14 6	150	424	0 75	10 1	165	77
D J	44	71	15	15	2	13 3	114	305	0 65	9 2	60	
E E	55	70	20			12 3	100		0 90	12 9	35	
F L	48	55	10			12 8	80	322	0 70	12 7	90	
F L. G	35	67	5			12 4			0 72	10 7		130
G S	57	61	10			13 2	117	298	0 84	13 8	60	128
H Sg	30	82	10			12 9	(142)	(409)	1 20	14 6	35	107
H E.	68	80	30	2	3	13 1	(54)	(47)	0 71	8 9	110	
H J	49	80	20			13 7	214	525	0 76	9 5	63	
H G	61	72	5			13 2	(143)	(270)	0 68	9 4	50	
H S	63	60	30	7	3	13 6			0 25	4 2	37	
J F	62	75	0			12 7	83	383	0 82	10 9	90	
J L	35	74	10			15 2	269	328	0 57	7 7		168
K R	49	76	10			15 1			0 82	10 8		
K G	69	53	30			14 4	153	377	0 49	9 2		
K C	55	72	20			15 7	189	390	0 83	11 5	54	)6
M K	74	68	10			11 9	63	383	0 83	12 2		179
L S	46	67	10			13 8	188	461	0 80	11 9	60	
L. Go	50	70	25	3	5	12 9	156	406	0 72	10 3	55	164
L N	54	68	20	3	5	12 5	(113)		0 79	11 6		48
M E	58	63	35	3	5	12 1			0 59	9 4	25	117
M S	55	86	30			15 2			0 61	7 1	105	
N O	34	87	5			12 3	104	345	0 45	5 2	30	
O Ae	40	88	5			12 1	69	415	0 48	5 5	25	
O Ar	47	99	15			13 6	146	345	0 56	5 7	35	
O H	56	78	15			12 5	(132)	(240)	0 62	7 9	55	241
O R	37	72	10			13 5	76	290	0 79	11 0		
S K	41	60	10			13 6	9	406	0 22	3 7	28	
S B	34	60	15			14 4	102	317	0 70	11 7	50	
S H	42	76	15			14 3	213	3 6	0 60	7 9		118
St S	56	72	25	14	3	12.4	71	281	0 80	11 1	50	
S So	29	90	5	2	2	14 3	182	308	0 58	6 4	90	
S E	63	75	10			13 2	173	360	0 69	9 2	0	158
S S	58	70	0			13 6			0 32	4 6	50	66
T L	43	114	5			15 0	2 0	461	1 59	13 9	80	117
V O	36	80	10	2	5	14 2	116	384	0 96	12 0	95	
V H	48	65	10	7	3	14 3			0 42	6 5	55	126
W T	6	62	20	2	5	12 3	128	295	0 85	13 7	34	14
W K	37	65	10			14 7	127	356	0 86	13 2	53	
Ö H	3	73	5			14 8	182	377	0 89	1 2	38	
Al ohol abusers w th DF indu~ed urinary iron excretion above the normal range												
An O	55	72	5			14 2			? 16	30 0	70	
F S	41	70	10			14 6	300	360	1 21	17 3	130	187
Fr S	44	67	5		2	14 5	217	298	1 08	16 1	163	
J C	31	65	5			13 1	103	304	1 34	0 6	30	
K J	60	89	5	7	5	12 1	73	326	1 55	17 4	65	138
S P	4)	75	10	3	5	13 9	115	281	1 54	70 5	100	287
Z S	57	75	10			12 5	(71)	(257)	1 24	16 5	60	
Å H	70	70	30			12 5	(86)	(299)	1 09	15 6	60	87

Table IV *DF induced iron excretion on two DF tests in 15 alcoholics with declining SGOT levels (Alcohol abusers group II)*

	SGOT				DF induced urinary iron excretion				Serum iron			
			Decrease				Decrease				Decrease	
Pat	1st test (units)	2nd test (units)	(units)	( )	1st test (μg kg)	2nd test (μg/kg)	(μg/kg)	( )	1st test (μg/100 ml)	2nd test (μg/100 ml)	(μg/100 ml)	( )
W K	730	53	677	93	16 3	13 2	3 1	19	132	127	5	4
O Ae	78	25	53	68	10 8	5 5	5 3	49	171	69	52	43
S H	70	30	40	57	7 7	6 9	0 8	10	70	56	14	[illegible]0
L Go	105	55	50	48	15 3	10 3	5 0	33	156			
Ba G	93	50	43	46	11 4	5 7	5 7	50	117	98	19	16
Sm E	100	55	45	45	17 9	16 3	1 6	9	147	107	40	27
M R	100	55	45	45	12 8	7 7	5 1	40				
D S	171	68	53	44	32 2	11 5	20 7	64				
H S	63	37	26	41	10 6	4 2	6 4	60				
E E	58	35	23	40	15 1	12 9	2 2	15	153	100	53	35
O Ar	54	35	19	35	10 3	5 7	4 6	45	177	146	31	18
T G	38	25	13	34	10 3	7 9	2 4	23	180	81	99	55
An L	50	37	13	26	11 3	7 8	3 5	31	150			
H J	75	63	12	16	9 0	9 5	−0 5	−16	171	214	−43	−25
F L	105	90	15	14	14 2	12 7	1 5	11	125	80	45	36
Mean ± s ε	123±44	48±5	75±43	43±5	13 7±1 5	9 2±0 9	4 5±1 3	30±6	142±9	108±15	32±1[illegible]	[illegible]3±7

steatosis (0 4 of visible fat) Beer consumption was low in all controls

The DF test was performed within three days before the operation The liver biopsy was performed at the beginning of the operation as previously described (6)

Alcohol Abusers

Most of the patients were alcoholics in the medical sense and all but four had been admitted to mental hospitals psychiatric wards or alcoholic institutions At the time of the investigation 80 of the patients were in a mental hospital (Clinic IV Lillhagen s Hospital) for chronic or acute alcoholism The rest were in a somatic ward (Department of Medicine I Sahlgren s Hospital) The causes of admission for the latter were in most patients sequelae of alcoholism

Only patients with a mean alcohol consumption converted to 40 ethyl alcohol of at least 1 litres per week for at least five years were included The figures for the duration of severe alcohol abuse in Table III are minimum values and probably underestimated in most cases. Wine consumption of one bottle per week was common but only 16 had consumed two or more bottles of wine per week for a period of more than a year (Table III) The latter consumed distilled alcoholic beverages as well. The consumption of distilled alcoholic beverages averaged seven litres per month Beer consumption was moderate in most patients

Alcohol abusers group I (Table III)

The group comprised 58 patients. Only subjects without a history of hemorrhage iron medication or blood transfusion were included Patients with a history of pancreatitis cirrhosis of the liver or gastric resection were excluded The DF test was usually performed one to two weeks after admission In most subjects the SGOT level was increased at the time of the test. Non hemin iron concentration in liver biopsy specimens was determined in 26 of these 58 patients

Alcohol abusers group II (Table IV)

In this group comprising 15 alcoholics with increased SGOT level at admission the DF test was performed twice The SGOT was lower at the second test. These subjects usually had consumed large quantities of alcohol the days before admission Eleven of these patients are also included in group I of alcohol abusers Four are not included in group I because of cirrhosis of the liver (D S and Sm E) gastric resection (M R) or blood donations (T G)

METHODS

The desferrioxamine test was performed as follows 500 mg of desferrioxamine B methanesulphonate was dissolved in 5 ml of sterile water and administered in a dosage of 10 mg per kg body weight Approximately half of the solution was given intragluteally on one side and the rest on the other side To prevent pain and a cumulation of the solution in a small muscular volume the injection needle was withdrawn about two cm during the injection The urine was then collected for 24 hours in iron free polyethylene bottles.

The method for determination of ferrioxamine iron in urine is based on that of Keberle (4) and modified ac

Table V *Means standard errors of mean and ranges of the controls and alcohol abusers group I*

	Controls, group I				Controls group II				Alcohol abusers group I			
	n	Mean	S E of mean	Range	*n*	Mean	S E of mean	Range	*n*	Mean	S E of mean	Range
Age	26	37 7	2 7	21–60	13	45 5	5 3	19 81	58	48 7	1 5	[illegible]4–74
Weight (kg)	26	76 8	1 8	58–105	13	77 5	3 4	61–99	58	72 9	1 5	53–114
Hemoglobin (g/100 ml)	6	14 6	0 1	13 5–16 4	13	14 3	0 2	13 5–15 4	58	13 4	0 1	11 0–15 7
Serum iron (μg/100 ml)	26	131	7	72–225	13	1?8	1?	36–19?	50	129	8	54–300
Transferrin (μg/100 ml)	?6	331	9	?64–417	12	353	11	281–400	48	350	10	40– 48
DF induced urinary iron excretion (mg)	26	0 78	0 04	0 47–1 35	13	0 81	0 07	0 49 1.24	58	0 80	0 05	0 2 ? 16
DF induced urinary iron excretion (μg kg body weight)	26	10 1	0 4	6 9–14 9	13	10 4	0 8	7 0–15 2	58	11 0	0 6	3 7–30 0

cording to Lundvall and Weinfeld (5) The error of a single determination calculated from 58 random duplicate determinations was 3 9% The iron determinations were performed in duplicate The results are given in μg of DF induced 24 hour urinary iron excretion per kg body weight and in mg per 24 hours

The liver function tests and the methods used for the determination of hemoglobin, serum iron iron binding capacity non hemin iron and quantification of fat in aspiration liver biopsy specimens were those previously described (6)

RESULTS

Control group I (Tables I V)

The mean DF induced 24-hours urinary iron excretion in the 26 subjects was $0\ 78 \pm 0\ 04$ with a range of 0 47 to 1 35 mg The mean iron excretion per kg body weight was $10\ 1 \pm 0\ 4$ μg with a range of 6 9 to 14 9 μg

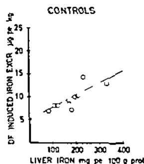


Fig 1 Relation between DF-induced iron excretion per kg body weight and non hemin liver iron in controls. The equation of linear regress on of the iron excretion upon liver iron concentration was $y = 0\ 027x + 5\ 1$ The correlation coefficient (0 70) was significant ($t = 3\ 26$ $p < 0\ 01$)

Control group II (Tables II V Fig 1)

The mean DF induced 24-hour urinary iron excretion in the 13 subjects was $0\ 81 \pm 0\ 07$ mg with a range of 0 49 to 1 24 mg The mean iron excretion per kg body weight was $10\ 4 \pm 0\ 8$ with a range of 7 0 to 15 2 μg The means of the two control groups were not statistically significantly different

The mean non hemin iron concentration in the liver biopsy specimens was $199\ 1 \pm 21\ 0$ with a range of 86 to 333 mg per 100 g protein

The relationship between DF induced iron excretion per kg body weight and liver iron concentration is shown in Fig 1 A correlation coefficient of 0 70 was obtained which was significant ($t = 3\ 26$ $p < 0\ 01$) The equation of linear regression of the iron excretion upon liver iron

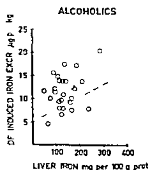


Fig 2 Relation between DF induced iron excretion per kg body weight and non hemin liver iron in alcoholics. The correlation coefficient for the regression of the iron excretion upon the liver iron concentration (0 35) was not significant ($t = 1\ 8$ $0\ 10 > p > 0\ 05$) The dotted line represents the regression line of the controls Most of the alcoholics fell above this line

concentration was $y = 0.027x + 5.1$ The correlation coefficient for the relation between DF induced urinary iron excretion not related to unit body weight and liver iron concentration ($r = 0.53$) did not reach the level of significance ($t = 2.1$ $0.10 > p > 0.05$)

Alcohol abusers group I (Tables III V Fig 2)

When more than one test was performed in the same patient only that test in which the SGOT level was lowest was included The mean DF induced 24-hour urinary iron excretion in 58 subjects was 0.80 ± 0.05 mg with a range of 0.22 to 2.16 mg The mean DF induced urinary iron excretion per kg body weight was 11.0 ± 0.6 μg with a range of 3.7 to 30.0 μg These means were not statistically different from either of the two control groups However values above the normal range (14.9 μg per kg body weight) were present in eight alcohol abusers They are grouped at the foot of Table III

The mean DF induced iron excretion of alcohol abusers who had consumed two or more bottles of wine per week for at least two years (11.3 ± 1.1 μg per kg body weight) was not significantly different from that of the others (10.9 ± 0.7 μg per kg body weight)

In 26 of the alcohol abusers non hemin iron was determined in liver biopsy specimens The mean non hemin iron was 139.0 ± 10.5 mg per 100 g protein with a range of 48 to 287 mg

The relationship between liver iron concentration and DF induced iron excretion per kg body weight in alcohol abusers is shown in Fig 2 The correlation coefficient ($r = 0.35$) was not significant ($t = 1.8$ $0.10 > p > 0.05$)

Alcohol abusers group II (Table IV)

In this group comprising 15 subjects with a declining SGOT level during the time of hospitalization the DF test was performed twice The first test was usually done 2–4 days after admission and the second 7–11 days later The mean SGOT concentration at the first test was 123 ± 44 units and at the second test 48 ± 5 units The DF induced iron excretion was lower at the second test in all but one (H J) The mean iron excretion at the second test was 30% lower The difference between the two tests was significant ($t = 3.54$ $p < 0.005$) The serum iron level was also determined in ten subjects at the time of both tests The mean serum iron level was significantly lower ($t = 2.68$ $p < 0.05$) at the second test The serum iron level was lower at the second test in all but one (H J) who also had a higher DF induced iron excretion at the time of the second test

DISCUSSION

In an earlier study (6) it was shown that alcohol abusers in Goteborg consuming predominantly distilled alcoholic beverages did not have increased liver iron stores as compared with a control group In the present study the mean DF induced iron excretion of 58 alcoholic men was not statistically different from that of a group of 26 normal men In a control group there was a significant correlation between the DF induced iron excretion per kg body weight and the liver iron concentration This is in accordance with results reported by Hallberg et al (3) In the alcohol abusers however no such significant correlation was obtained The DF induced iron excretion in the latter usually exceeded the amount predicted from their liver iron concentration In eight alcoholics the iron excretion exceeded the normal range In hepatitis increased DF induced iron excretion was found during the acute phase (9) To study whether the same was the case in alcoholic liver injury the test was performed soon after admission and then 7–11 days later It was found that the mean DF induced iron excretion was significantly lower at the second test when the SGOT level had decreased The mean serum iron was also lower at the second test It therefore seems probable that the higher serum iron level and greater DF induced iron excretion at the first test were caused by active liver injury

Liver biopsy was performed in five of the eight alcoholics whose iron excretion was above the normal range In four chemically determined non hemin iron was within the range of the controls In the fifth (An O) only histochemical estimation was performed and showed a grade of 2+ in parenchymal cells a common finding in controls (6) The SGOT level was considerably increased in two others with a moderate increase in DF induced iron excretion In one patient (J C) however the test was performed one month after admission and the SGOT concentration was

no ~al It is possible that in this patient the moderately increased iron excretion indicated in creased iron stores

Thus the mean DF induced urinary iron excre tion of the alcohol abusers was not different from that of controls In some alcohol abus~rs the DF induced iron excretion was increased as compared with controls This increase was probabl) due to enhanced DF-chelation of iron caused by hepatic cell injury and not to enlarged iron stores

ACKNOWLEDGEMENT

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THE BEHAVIOUR OF CERULOPLASMIN FRACTIONS IN LIVER DISEASES

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Abstract Ceruloplasmin fractions I(C–C) and II(C–D) have been estimated in patients with infectious hepatitis, liver metastases and cirrhosis of the liver In infectious hepatitis and metastases serum ceruloplasmin content was increased and the percentage of fraction I(C–C) was decreased no correlation between these parameters could be found. In the group of cirrhotics mean serum ceruloplasmin concentration was normal but the mean percentage of fraction I(C–C) was decreased A positive correlation between these values could be demonstrated Very low ceruloplasmin levels were found in some patients with the most severe form of liver cirrhosis if this is the result of an impaired synthesis, it seems probable that especially fraction I(C–C) is affected by this disturbance The possibility that only fraction I(C–C) is synthesized by the liver is discussed.

As the copper-containing blue protein ceruloplasmin is synthesized by the liver (15 17 20 29 30) it is probable that diseases of this organ will be accompanied by alterations in its serum content This has been shown by many authors In cirrhosis of the liver high serum levels have been found (2 9 32 34 35) as well as normal (8 11 18 22 31 33) or low levels (8 11 27 28) The highest serum ceruloplasmin concentrations were seen in patients with biliary cirrhosis (11 22 32) Normal or slightly elevated levels were found in infectious hepatitis (1 8 12 13 16 27 31) but in subjects with acute liver atrophy the ceruloplasmin content may be low (34) Elevated serum ceruloplasmin concentrations have been found also in patients with bile duct obstruction (8 12 21 27) primary carcinoma of the liver (22 31) liver metastases (1 8 28) cholangitis (31) and congestion of the liver in cardiac failure (8)

Less attention has been paid to the behaviour of ceruloplasmin fractions in patients with liver disease Broman (3 4) was the first investigator to report that ceruloplasmin can be separated in fraction I (or C–C) and fraction II (or C–D) this has been confirmed by other investigators (5 6 14 19 24 25 26) The bulk of the serum ceruloplasmin consists of fraction I(C–C) 75–85% the remaining 15–25% is fraction II(C–D) The sense of these fractions is not clear Alterations in the ratio of the percentages have been reported in patients with Wilson s disease the nephrotic syndrome and in newborns (5 24 25 26) Buyze (5) found in one patient with infectious hepatitis and in one patient with cirrhosis of the liver normal percentages for both fractions

As we could detect no other report in the literature concerning ceruloplasmin fractions in liver diseases we decided to investigate whether alterations in liver function, due to some pathological condition, have any influence on the ratio of the two ceruloplasmin fractions if such an influence exists perhaps it may be possible to get some information about the relation between these fractions

MATERIAL AND METHODS

Twelve patients with infectious hepatitis, 17 with liver metastases from malignant tumours and 37 with cirrhosis of the liver were studied. Data of age sex and results of liver function tests are given in Tables I, II and III respectively

In the infectious hepatitis group the diagnosis was based on the clinical picture and laboratory findings only in patient 1 was a percutaneous liver biopsy taken. Tumour metastases were established in all patients by autopsy liver biopsy or liver scintigraphy except in patients 9 11 and 14 in whom biopsy was not allowed because of disturbances of the clotting mechanism in the latter cases a proven primary tumour an enlarged and nodular liver and abnormal liver function tests made the diagnosis completely obvious.

Table I *Infectious hepatitis*

Pat no	Sex	Age	Ceruloplasmin (mg 100 ml serum)	Copper (μg 100 ml serum)	Ceruloplasmin fraction I (C–C) ( )	Ceruloplasmin fraction II (C–D) ( )	Bilirubin (mg 100 ml)	Alk phosph[a] (U)	Thymol t.t[b] (U)	SGOT[c] (U)	SGPT[d] (U)
Normal values			18 9 33 7	72 6–1?4 4	84 1–89 3	10 7–15 9	<1 0	0 8 2 3	<5	5–40	5–35
1	o	42	3? 3	121 1	78 3	21 7	5 5	6 8	13 2	164	350
2	o	20	.6 5	102.8	79 2	20 8	10 6	4 7	19 4	143	180
3	o	15	?1 3	80 5	86 1	13 9	2.3	5 2	18 6	54	140
4	o	78	36 4	142 1	87 8	12 2	3 9	2.8	6 0	>400	>400
5	o	4?	32 9	119 1	80 9	19 1	1 9	2 6	5 7	.36	>400
6	♂	14	34 7	128 2	75 7	24 3	18 5	8 4	27 4	>400	>400
7	o	29	32 3	114 0	75 1	24 9	1 6	6 7	7 3	137	153
8		17	25 9	95 6	84 1	15 9	1 0	3 5	13 4	24	95
9	o	42	52.9	192 2	79 5	.0 5	. 4	7 8	9 3	160	14
10	o	6?	23 8	87 3	79 3	20 2	6 9	6 8	15 2	400	640
11		56	55 3	02.8	78 3	21 7	3..3	3 8	34 0	440	630
12		63	42 4	156 5	83 6	16 4	4 7	6 2	22 0	3?0	940

[a] Alk phosph = alkaline phosphatase Bessy units
[b] Thymol t t = thymol turbidity test
SGOT = serum glutamic oxalacetic transaminase
[d] SGPT = serum glutamic pyruvic transaminase
[e] LDH = lactic dehydrogenase

In 27 patients with cirrhosis the diagnosis was made on histological grounds In ten patients a liver biopsy could not be performed because of severe thrombocytopenia or a disturbed clotting mechanism however all of them had an elevated hepatic vein wedged pressure and portal systemic collaterals besides a suspected history clinical signs and laboratory findings The ceruloplasmin fractions were estimated in 20 cirrhotics only

Serum ceruloplasmin was estimated by a modified chromatographic-spectrophotometric method (7 10) Se

Table II *Liver metastases*

Pat no	Sex	Age	Primary tumour	Ceruloplasmin (mg 100 ml serum)	Copper (μg/100 ml serum)	Ceruloplasmin fraction I (C–C) ( )	Ceruloplasmin fraction II (C D) ( )
Normal values				18 9–33 7	72 6–124 4	84 1 89 3	10 7 15 9
1		51	Renal carcinoma	42 6	153 4	79 8	0 2
2		60	Thyroid carcinoma	28 8	105 .	87 6	1? 4
3	o	26	M Hodgkin	35 3	1 9 4	70 0	30 0
4		46	M Hodgkin	43 3	162 6	88 1	11 9
5		81	Renal carcinoma	44 8	162 7	92.4	7 6
6		80	Pancreatic carcinoma	61 0	?19 7	80 2	19 8
7		67	Melanoblastoma	46 3	167 9	89 9	10 1
8	o	48	Bronchial carcinoma	37 0	116 5	83 3	16 7
9	♀	76	Pancreatic carcinoma	40 6	15? 3	80.3	19 7
10	o	63	Bronchial carcinoma	.9 4	113 2	83 2	16 8
11	♀	61	Melanosarcoma	35 3	131 7	81 4	18 6
12	♀	6	Gallbladder carcinoma	42 9	154 1	84 7	15 3
13	o	73	Carcinoma of the stomach	.7 0	101 2	79 6	.0 4
14	o	56	Carcinoma of the stomach	48 ?	18? 1	81 9	18 1
15	o	57	Carcinoma of the sigmoid	32.9	111 2	76 7	.3 3
16	o	63	Gallbladder carcinoma	45 1	165 9	78 4	21 6
17	o	53	Bronchial carcinoma	47 6	173 2	83 1	16 9

[a] Alk phosph = alkaline phosphatase Bessy units
[b] Thymol t t = thymol turbidity test
[c] SGOT = serum glutamic oxalacetic transaminase
[d] SGPT = serum glutamic pyruvic transaminase
[e] LDH = lactic dehydrogenase
[f] BSP retention = retention of bromsulphalein (5 mg kg body weight) after 45 min

LDH (U)	Cholesterol (mg 100 ml)	Serum protein (g 100 ml)	Serum albumin (g/100 ml)	Serum globulin (g/100 ml)
150-350	180–280	6 0–8 0	3 6–4 8	2 4–3 2
335	—	7 70	3 42	4 28
345	—	7 70	4 12	3 58
180	196	7 0	3 07	4 13
1240	280	7 20	3 02	4 18
470	—	6 10	4 08	2.02
585	158	11 10	5 30	5 80
830	140	6 83	3 12	3 71
6 0	133	7 80	3 98	3 82
580	147	7 34	3 61	3 73
360	274	7 69	3 24	4 45
450	199	7 99	4 12	3 87
460	234	7 12	3 18	3 94

rum copper determinations were done as described by Rice (3) *Separation of ceruloplasmin in fraction I(C–C)* and fraction II(C–D) was don by gradient-chromatography on hydroxylapatite columns according to Broman (3 4)

In 4 normal subjects the following mean values were found ceruloplasmin 63 mg/100 ml s rum (S D 3 7) fraction I(C–C) 86 7% (S D 1.3%) fraction II(C–D) 13 3 % (S.D 1.3%) and copper 98 4 μg/100 ml serum (S D 1 9) There were no significant differences in these values between both sexes.

RESULTS

(a) *Infectious hepatitis*

The concentrations of serum ceruloplasmin and serum copper and the percentages of the two fractions are given in Table I Both the mean ceruloplasmin (34 7 mg/100 ml S D 10 8) and the mean copper (125 2 μg/100 ml S D 38 9) concentrations are significantly higher than in normals ($p < 0.005$) The mean value for fraction I(C–C) is 80 7% (S D 3 8) and for fraction II (C–D) 19 3% (S D 3 8) fraction I(C–C) is significantly lower and fraction II(C–D) significantly higher than in normal subjects ($p < 0.005$)

There is no correlation b tween ceruloplasmin contents and the percentages of the fractions ($p > 0.05$) Considering the liver function test results a positive correlation only between serum ceruloplasmin content and SGOT is found ($0.05 > p > 0.025$)

Bilirubin (mg/100 ml serum)	Alk phosph (U)	Thymol t t [b] (U)	SGOT (U)	SGPT [d] (U)	LDH (U)	BSP retention [f] ( )	Serum protein (g 100 ml)	Serum albumin (g/100 ml)	Serum globulin (g/100 ml)
1 0	0 8–2 3	< 5	5–40	5 35	150–350	< 5	6 0–8 0	3 6–4 8	2 4–3
—	3 9	1 4	120	73	—	30	7 12	2 46	4 66
—	5 8	0 6	—	—	155	25	6 04	3 34	70
7 6	15 4	1 4	174	78	215	—	6 82	2.66	4 16
2 9	24 9	1 9	48	0	340	80	6 05	3 86	2 19
—	2.7	3 5	—	—	—	—	6 40	3 12	3 8
10 2	21 5	1 4	58	58	435	80	6 60	2.43	4 17
1 0	3 2	4 0	13	16	300	—	6 40	4 50	1 90
—	14 2	0 9	61	60	—	30	7 93	2.87	5 06
3 2	14 0	0	136	128	290	9	—	—	—
1 0	10 1	0 3	08	23	815	30	6 72	3 60	3 12
1 0	6 0	0 2	134	127	350	50	6 01	3 58	2 43
—	4 7	0 5	15	15	—	12.5	6 32	3 05	3 27
1 0	3 7	1 0	61	68	—	40	5 54	2.75	2 79
< 1 0	11 2	0 5	80	72	11 0	—	—	—	—
1 0	6 6	1 7	—	—	395	7 5	5 63	—	—
< 1 0	18 0	0 9	130	52	1970	90	6 48	3 21	3 27
—	2.6	6	46	58	80	31	3 50	1 4	2.08

Table III Liver cirrhosis

Pat no	Sex	Age	Type of cirrhosis	Ceruloplasmin (mg 100 ml serum)	Copper (μg 100 ml serum)	Ceruloplasmin fraction I (C-C) ( )	Ceruloplasmin fraction II (C-D) (*)
Normal values				18 9-33 7	72.6-124 2	84 1 89 3	10 7-15 9
1	♂	31	Alcoholic	29 4	137 1	86 5	13 5
2	♀	59	Alcoholic	24 7	91 3	—	—
3	♂	53	Alcoholic	37 5	135 8	—	—
4	♂	64	Alcoholic	27 5	99 6	—	—
5	♀	47	Alcoholic	25 8	94 9	84 8	15 2
6	♂	43	Alcoholic	14 7	53 4	80 8	19 2
7	♀	69	Alcoholic	22 7	83 6	83 5	16 5
8	♂	36	Alcoholic	29 1	108 1	—	—
9		39	Post hepatitic	12 2	47 1	75 1	24 9
10	♂	37	Post hepatitic	17 6	73 1	81 5	18 5
11	♀	69	Post hepatitic	36 3	131 3	—	—
12	♀	62	Post hepatitic	30 6	113 7	86 5	13 5
13	♂	39	Post hepatitic	28 3	104 6	88 4	11 6
14	♂	59	Post hepatitic	32 3	119 4	85 2	14 8
15	♂	65	Post hepatitic	22 3	84 5	83 5	16 5
16	♀	79	Post hepatitic	23 7	88 6	84 4	15 6
17		49	Post hepatitic	20 0	74 8	—	—
18		6	Biliary	64 7	247 3	—	—
19		73	Biliary	43 8	169 2	—	—
20	♂	54	Biliary	47 6	180 5	—	—
21		70	Biliary	37 3	134 4	—	—
22	♂	64	Cryptogenic	40 0	146 2	88 3	11 7
23		75	Cryptogenic	19 2	73 1	80 8	19 2
24	♂	50	Cryptogenic	28 8	106 2	—	—
25	♂	76	Cryptogenic	38 2	137 1	—	—
26	♂	73	Cryptogenic	24 1	89 4	—	—
27	♂	76	Cryptogenic	23 5	86 1	—	—
28		54	Cryptogenic	22 4	81 5	82 7	17 3
29	♂	39	Cryptogenic	12 9	50 1	—	—
30	♂	38	Cryptogenic	16 7	66 1	77 6	22 4
31		47	Cryptogenic	33 3	125 4	86 9	13 1
32		61	Cryptogenic	39 5	148 5	83 0	17 0
33	♂	60	Cryptogenic	12 4	48 8	76 4	3 6
34		68	Cryptogenic	45 7	157 9	85 1	14 9
35	♂	43	Cryptogenic	19 6	73 8	81 7	18 3
36		66	Cryptogenic	18 8	69 2	—	—
37		63	Cryptogenic	28 ?	103 5	—	—

[a] Alk phosph alkaline phosphatase Bessy units
[b] Thymol t.t = thymol turbidity test
[c] SGOT serum glutamic oxalacetic transaminase
[d] SGPT = serum glutamic pyruvic transaminase
[e] LDH = lactic dehydrogenase
[f] BSP retention = retention of bromsulphalein (5 mg kg body weight) after 45 min

(b) *Liver metastases*

Table II presents the values for ceruloplasmin copper and the percentages of the fractions in this group of patients The mean ceruloplasmin concentration is 40 5 mg/100 ml serum (S D 8 6) the mean copper concentration 148 1 μg/100 ml serum (S D 32 7) the mean percentage of fraction I(C–C) is 82 4 % (S D 5 3) and of fraction II(C–D) 17 6 % (S D 5 3)

In comparison with the same values in healthy individuals serum ceruloplasmin serum copper and fraction II(C–D) are significantly higher and fraction I(C–C) significantly lower ($p < 0\ 005$)

No correlation exists between ceruloplasmin concentrations and the percentages of the fractions ($p > 0\ 05$)

Only between SGOT on the one hand and ceruloplasmin concentration and the percentage

Bilirubin (mg/100 ml serum)	Alk phosph (U)	Thymol t.t [b] (U)	SGOT (U)	SGPT [d] (U)	LDH (U)	BSP retention [f] ( )	Serum protein (g/100 ml)	Serum albumin (g/100 ml)	Serum globulin (g/100 ml)
<1 0	0 8–2 3	<5	5–40	5–35	150–350	<5	6 0–8 0	3 6–4 8	2 4–3 2
1 0	6 7	1 0	29	6	480	12 5	7 76	3 65	4 11
3 ?5	2 7	8 7	50	25	.65	60	7 80	3 31	4 49
2 2	3 3	1 6	70	50	453	60	7 96	3 95	4 01
17 1	3 2	11 3	112	72	.45	70	7 41	2.45	4 96
1	2 3	4 8	12	14	170	100	7 25	2 96	4 29
1 5	3 5	3 7	34	16	405	15	5 05	2 55	2 50
—	2 0	0 5	3	8	160	5	6 89	3 34	3 55
<1 0	2 6	4 0	23	26	270	3	8 02	4 49	3 53
2 1	2 3	12 8	49	46	355	25	6 60	3 37	3 23
1 0	4 3	2 8	39	30	280	3	8 13	4 42	3 71
1 1	3 6	4 4	137	107	270	50	6 06	2 63	3 43
<1 0	5 8	11 8	350	94	—	17	9 66	3 72	5 94
<1 0	4 8	20 4	186	220	210	40	8 75	4 83	3 92
<1 0	1 8	3 9	4	8	?40	15	6 81	3 94	2 87
4 2	3 2	14 4	196	104	420	70	6 92	2 80	4 12
<1 0	2 1	2 7	22	14	280	12 5	6 32	3 86	2 46
<1 0	9 9	2 2	16	42	220	7	6 30	3 58	2.72
9 3	10 8	1 4	146	56	230	40	6 42	2 87	3 55
15 6	5 6	3 9	90	38	235	70	7 00	2 75	4 25
5 5	19 0	4 3	51	47	—	—	7 35	2.65	4 70
2 5	12 5	4 4	83	110	280	35	7 89	3 31	4 58
1 0	2.5	1 4	23	9	290	3	8 .8	4 42	3 86
—	5 8	6 6	13	9	290	7	6 68	3 88	2 80
<1 0	15 4	5 5	63	83	230	10	7 54	3 82	3 7.
4 5	4 4	14 4	760	640	550	70	9 30	1 86	7 44
1 2	2.5	7 4	54	40	345	70	6 60	2 33	4 27
<1 0	3 .	3 1	41	24	440	10	5 42	3 52	1 90
<1 0	2 8	1 0	29	39	250	10	6 60	3 72	2 88
1 ?	2 1	2 9	26	13	235	20	6 58	3 11	3 47
<1 0	1 9	3 6	16	4	190	3	7 24	4 82	2.42
<1 0	2 7	0 8	21	10	390	23	6 00	3 00	3 00
<1 0	2 1	7 5	16	5	470	16	6 47	2 40	4 07
<1 0	3 6	2 6	30	14	3 0	40	6 65	3 80	2 85
1 3	2 7	14 2	58	38	180	23	8 38	4.32	4 06
<1 0	1 9	3 8	13	9	180	<3	6 55	4 50	2 05
5 0	4 8	9 0	126	170	—	—	6 00	2 31	3 69
<1 0	2 9	8 6	?5	16	370	35	8 66	4 05	4 61

of fraction I(C–C) on the other is a negative correlation found (0 05 > p > 0 025 and 0 025 > p > 0 01 respectively)

(c) *Cirrhosis of the liver*

Data of ceruloplasmin copper and ceruloplasmin fractions are given in Table III

In the whole group of patients the mean serum ceruloplasmin level (28 4 mg/100 ml S D 10 2) and the mean copper content (106 6 μg/100 ml S D 42 2) do not differ from the normals (p > 0 05)

In the group of alcoholic cirrhosis the mean ceruloplasmin concentration is 26 3 mg/100 ml (S D 6 6) and the mean serum copper content 97 9 μg/100 ml (S D 24 3) These values in the group with post hepatitic cirrhosis are 24 8 mg/100 ml (S D 7 7) and 92 7 μg/100 ml (S D 26 7) in the group of biliary cirrhosis 48 3 mg/100 ml (S D 11 7) and 182 8 μg/100 ml (S D 47 2) and in the group of cryptogenic cirrhosis 26 5 mg/100 ml (S D 10 2) and 99 8 μg/100 ml (S D 37 5)

Only in the group of biliary cirrhosis does a significant difference exist in serum ceruloplasmin and copper contents in comparison with the normal values (p < 0 005) In the whole patient group a positive correlation is found between

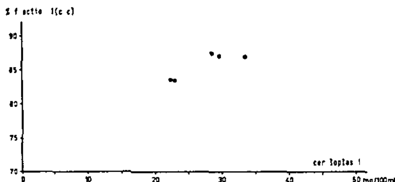


Fig 1 The serum ceruloplasmin concentrations plotted against the percentages of fraction I(C–C) in 20 patients with cirrhosis of the liver

the ceruloplasmin concentration and both the serum total protein ($0.05 > p > 0.025$) and serum globulin content ($0.01 > p > 0.005$)

In the group of alcoholics a positive correlation is found only between serum ceruloplasmin and total serum protein ($0.05 > p > 0.025$) In none of the other groups is there a correlation with any of the liver function tests

In 20 patients the fractions were estimated without respect to the aetiology of the cirrhosis The mean percentage of fraction I(C–C) is 83 1 % (S D 3 8) and of fraction II(C–D) 16 9 % (S D 3 8) In comparison with the same values in normal subjects fraction I(C–C) is significantly lower and fraction II(C–D) significantly higher ($p < 0.005$) There is no correlation with any of the liver function tests A positive correlation exists between serum ceruloplasmin concentrations and the percentages of fraction I(C–C) ($p < 0.005$ Fig 1)

DISCUSSION

With regard to the serum ceruloplasmin and serum copper concentrations our results are to a great extent in conformity with the data published by other authors Elevated levels were seen in nearly all patients with liver metastases and normal or slightly elevated levels in patients with infectious hepatitis most patients with cirrhosis of the liver had normal values with the exception of the patients with a biliary cirrhosis all of whom had considerably elevated levels

However a few cirrhotic patients had ceruloplasmin concentrations lower than in normal subjects this is in accordance with the observations of Rotelli (27) Gault (8) and Secchi (28) They also found a low ceruloplasmin content in some patients especially in those with a very severe cirrhosis The explanation of this result was a disturbed synthesis of ceruloplasmin by the liver Gault (8) observed fluctuations in serum ceruloplasmin content corresponding to the degree of severity of the illness of the patient he found a lowering of the concentration when the clinical picture became worse We also found the lowest serum ceruloplasmin concentrations in the cirrhotic patients who had the most marked disfunction of the liver

In the group of patients with infectious hepatitis and liver metastases a lowering of the percentages of fraction I(C–C) in combination with an elevated serum ceruloplasmin content was observed Nevertheless correlation between these values is lacking so it cannot be concluded that the elevation of the ceruloplasmin concentration depends on an increase of one fraction or another Because there is a correlation only between ceruloplasmin and SGOT liver function tests do not add to our understanding of this problem

The observation that in the group of patients with cirrhosis the ceruloplasmin concentrations correlate positively with total serum protein contents and with serum globulin contents may be an argument for the supposition that the low ceruloplasmin content as seen in some patients is due to a disturbed synthesis

Viewed in the light of the positive correlation between serum ceruloplasmin concentrations and the percentages of fraction I(C–C) it is attractive to assume that a decrease of serum ceruloplasmin is effected at the cost of fraction I(C–C) However if the calculated quantitative values (i e in mg/100 ml) of the fractions in all 20 patients are compared with the same values in normal sub-

jects it becomes evident that fraction I(C–C) is not smaller but that fraction II(C–D) has increased The proportional decrease of fraction I(C–C) is the result of a real increase of fraction II(C–D)

Still this observation does not explain the positive correlation between serum ceruloplasmin concentrations and the percentages of fraction I(C–C) For instance when the proportional decrease of fraction I(C–C) is only the result of a real increase of fraction II(C–D) we might expect the highest values for fraction II(C–D) in cases in which the percentages of fraction I(C–C) are very low i e in the patients with the lowest serum ceruloplasmin levels The overall mean for fraction II(C–D) *is 4 0 mg/100 ml (S D 1 1) this is* significantly higher than in normals ($p < 0.005$) In the patients with a low serum ceruloplasmin content (< 18.9 mg/100 ml) the mean real fraction II(C–D) is 3 2 mg/100 ml (S D 0 5) which does not differ significantly from the same mean in normals ($p > 0.05$) in these cases the decrease *of serum ceruloplasmin is caused by a real de*crease of fraction I(C–C)

In other words low ceruloplasmin levels in cirrhotic patients are due to a decrease of fraction I(C–C) while fraction II(C–D) is not altered When these low ceruloplasmin levels are the result of a decreased synthesis no other conclusion can be drawn than that this impaired synthesis affects especially fraction I(C–C) If serum ceruloplasmin content is normal or elevated in patients with liver cirrhosis the real value of fraction II(C–D) is increased

One explanation of these observations may be found in the hypothesis that only fraction I(C–C) *is* synthesized by the liver and that fraction II(C–D) originates somewhere else When synthesis is disturbed fraction I(C–C) will decrease but fraction II(C–D) is able to remain unchanged The result is an altered ratio for the percentages of the fractions However this hypothesis does not answer the question why fraction II (C–D) is *increased* in cirrhotic patients with normal or elevated ceruloplasmin levels perhaps the same unknown factors are responsible which are operative in the changed ratios in patients with infectious hepatitis and tumour metastases At this moment our knowledge of the ceruloplasmin fractions is too small to explain all the observed alterations

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ON THE CONTENT OF CYTOCHROME C IN HUMAN MUSCLES

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Abstract A quantitative method of determining cytocrome *c* in human muscles is described.

A piece of muscle about 4 g, is ground with dry ice homogenized and exhaustively extracted with phosphate buffer of pH 7 4 containing 0 4 M sodium chloride After being centrifu ed at 15 000 × *g* the supernatant is acidi fed to pH 4 0 at which pH cytochrome *c* remains in solution and a large coloured precipitate is removed by centrifugation. The supernatant is neutralized and all re maining heme groups containing proteins exc pt cytochrome *c* are precipitated with ammonium sulphate at 60% saturation

The cytochrome *c* is then determined by spectrophotometry and calculated as mg per 100 g of dry weight, the latter being determined on the original homogenate

In an autopsy material mainly consisting of elderly patients, the cytochrome *c* content was found to be about 105 mg per 100 g dry muscle in the left ventricular myocardium 90 mg in the right ventricular myocardium 33 mg in the diaphragm and 18 mg in upper thigh muscles. The relation between h art and skeletal muscle was of the order of 6 1 The limited number of subjects stud ed does not permit any definite conclusions as regard the relation ship of the cytochrome *c* content to other biological or clinical parameters in the material.

The values obtained are higher than reported previously (about twice or more) whereas the relations between values *for heart, diaphragm* and other skeletal muscle seem to be quite similar to those given earlier Some comments are given to explain the differences.

It is thought that this method as well as that for myoglobin recently reported, may be of value for the study of the physiology and pathology of iron metabolism.

In a previous paper (1) we have reported our findings concerning the content of myoglobin in human muscles using a new method of deter mination of certain heme compounds in muscle At the outset it was our aim to find a procedure by means of which both myoglobin and cytochrome *c* could be determined simultaneously For reasons given in the paper this goal was not achieved However in the course of our work we had observed quantities of cytochrome *c* that ap peared to be larger than generally observed in previous studies (Table I) We th refore decided to pursue the course and study cytochrome *c* alone The technique which was worked out is briefly described below

MATERIAL

Determinations were performed on material derived from routine hospital autopsies. Muscle tissue was excis d from the apex of the left and right ventric es, the diaphragm, and musc es of the upper thigh Four g of muscle were used for every single analysis The muscle was stored at −80 C if it could not be analyzed immediately In all cases microscopical studies were performed on the muscle piece subjected to analysis. Some clinical and autopsy data are recorded in tables II and III It should be noted that no cases of acute myocardial infarction were us d for stud es on heart muscle Microscopy of skeletal muscles did not show any notable pathology Dr H H Nordenstam of the Department of Pathology prov ded the specimens and the m croscopical examinations

Apparatus and chemicals

All centrifugations were performed in an MSE super speed 50 S ultracentrifuge at a temperature of +4 C A Cary model 14 or a Beckman DK-2 A spectrophotometer with linear wavelength scale was used for recording spectra.

The all-glass homogenizer was of the Potter Elvehjem type with a total volume of 100 ml and a pestle dia meter of 20 mm. It was driven by a motor and the pestle speed was kept at 1500 rpm.

All chemicals were of analytical grade

METHODS

A p ece of muscle approximately 4 g, was d ss cted free of fat and connective tissue and dried carefully w th filter paper The muscle was then cut into smaller pieces,

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Table II *Some clinical data concerning the subjects examined*

Autopsy serial no	Sex	Age	Duration of illness: Acute	Duration of illness: < 1 month	Duration of illness: 1 month or more	Anemia: None	Anemia: Marked	Anemia: Not known
211/67	♀	57	+					+
266/67	♀	52	+			+		
308 67	♀	76	+					+
311/67	♀	76			+	+		
3?2 67	♀	74	+					+
333 67	♂	41			+	+		
344 67	♀	59	+					+
351/67	o	61		+		+		
357/67	♂	75			+	+		
361/67	♂	76			+	+		
375 67	♂	77			+			+
377 67	♂	98				+		
381/67	♂	54		+		+		
382 67	♂	66		+			+	
385 67	♂	62	+			+		
390 67	♀	56						+
39 67	♂	75				+		
398 67	o	67	+					+
405 67	♂	57					+	
406 67	♂	63	+					+
407 67	o	65			+	+		
19/68	o	47	+					+
21/68	o	69	+					+
40 68	♂	80	+					+
45 68	♂	78		+		+		
46 68	♀	85				+		
48 68	♂	64				+		
57/68	♀	73			+			
62 68	♂	69				+		
71/68	♂	56						+
75 68	o	43		+		+		
76/68	o	7			+			
80 68	♀	64			+		+	
84 68	o	86	+					+
99 68	♀	77	+					+
104 68	♀	48	+					
111 68	♀	88			+	+		
11 68	♀	84				+		
1?5 68	♂	60				+		
127/68	♀	79		+		+		
148 68	♀	82				+		
149 68	♂	55	+					+
150 68	♀	69	+					
151/68	o	55	+					+
166 68	♀	71	+					+
167 68	+	84				+		
169 68	o	60	+					+
17 68	♂	73				+		
173 68	♂	91		+		+		
184 68	♀	37			+		+	
18? 68	♀	81			+	+		
137 68	♀	81		+		+		

on the absence of other hemoproteins the maximum absorption of cytochrome *c* in this region being at 415.5 mμ. In no case was there however any indication of the presence of hemoproteins other than cytochrome *c*.

As there was no shortage of material in the present work, in which autopsy material was used, it was found convenient to work with 4 g of muscle for every single determination. This amount may however be decreased considerably without loss of accuracy to about 1.5 g for diaphragm and thigh muscle and to about 0.5 g for heart muscle.

Attempts were made to use the considerably higher

Table III *Content of cytochrome c in human myocardium*

Determinations on 4 g of muscle Brackets indicate duplicate analyses

Autopsy serial no	Cytochrome *c* mg/100 g dry weight[a] LV	RV	Heart weight	Distribution of fibrosis between LV and RV (+ + marked + some − uncertain)		Hypertrophy LV	RV
311/67	144 50	—	470 g			L	
308 67	115 12	100 00	495 g	L	+		R
311/67	91 00	57 27	370 g	L	+ +		
32? 67	76 33	79 28	360 g	L	+ +		
331 67	61 57	74 09	280 g	LR	−		
344 67	106 00	86 72	470 g	L	+	L	R
361/67	78 96	95 64	510 g	L	+	L	R
377 67	104 72	−	410 g				
381/67	137 40	—	570 g			L	
38 67}	104 45	—	325 g				
382 67}	105 00	—					
38 67}	96 86	—					
38? 67}	96 10	—					
385 67	116 03	—	440 g				
390 67	91 14	—	300 g				
392 67	119 79	73 92	725 g	L	+ +	L	
405 67	94 33	68 05	375 g	L			
406 67	11. 60	78 35	445 g	L	+	L	
407 67	87 88	81 99	380 g	L	+		R
19 68	1 4 40	12? 53	500 g	LR	−	L	
99 68}	84 15	—	450 g			L	
99 68}	90 40	—					
104 68}	123 66	96 51	460 g	LR	−	L	
104 68}	135 63	—					
151/68}	119 93	106 95	470 g	L	+		
151 68}	110 03	113 41					
17. 68	123 77	100 50	1030 g	L	+	L	R
173 68	112 40	10 30	700 g	L	+	L	R
184 68	111 03	93 31	300 g	LR	−		
186 68	1.0 03	88 36	4 0 g	L			R
187 68	107 45	101 39	290 g	L	+		R
	106 36 ± 3 41	90 56 ± 3 77					

[a] Mean values given as mean ± s.e. of mean

light absorption at 415 5 mμ for the determination These attempts failed however because of the very high extraneous light absorption in this region, necessitating a large and very uncertain correction

Flatmark (11) has described a method for assaying microquantities of soluble cytochrome *c* in homogenates of perfused rat kidneys This method which probably could be applied to other and unperfused tissues would further decrease the amount of tissue necessary for the determination. A lower limit is set, however by the necessity of dry weight determination in quantitative analysis.

RESULTS

The results obtained are presented in Tables III and IV It is seen that the cytochrome c content of the myocardium amounts to about 105 mg/100 g dry weight of muscle for the left ventricle and somewhat less for the right ventricle Values for diaphragm and thigh muscles are considerably lower about 33 and 18 mg per 100 g respectively The mean error of the duplicate determinations were found to be for heart muscle ± 4 0% for diaphragm ± 5 3% and for thigh muscle ± 3 7 The number of cases is too small to permit clinical conclusions The cytochrome *c* values in some eight cases in which right ventricular hypertrophy was observed at autopsy were slightly higher than in the other cases This is in accord with previous findings by Biorck (6 7 9) and Dallman (10) but the differences are probably not significant Despite high age and prolonged illness in many of the subjects studied anemia

was infrequent No apparent relationship b tween cytochrome *c* and hemoglobin values could be established in this series Cytochrome *c* values for heart and skeletal muscle were somewhat lower in patients dying after more than one months illness than in cases dying after only a short ill ness (Fig 1) The figures for cytochrome c in heart muscle (average) in comparison to those from skeletal muscle (thigh muscles) give a rela tion of about 6 1 The diaphragm seems to be higher in cytochrome *c* than thigh muscles

DISCUSSION

The data on the cytochrome *c* content of some human muscles presented above are higher than those previously reported (Table I) It is interest ing to note that Biorck in 1951 (5) noted that values arrived at by a preliminary method of K G Paul (13) had been more than twice as high as those obtained by the method of Rosenthal and Drabkin However previous observations on the distribution of cytochrome *c* between heart and skeletal muscle as well as between left and right ventricles and the somewhat intermediate position of the values for diaphragm have been further confirmed

We have carried out som comparisons of the present technique with that of Loftfield and Bon nichsen as employed in earlier work (6 7 8) The range of values arrived at by that method was found to be of the same ord r as in the earlier series (6 7 8) while simultaneous deter minations with the present method gave consider ably higher values Apart from the fact that in the meantime new figures have b en worked out for the molar light absorption and molecular weight of cytochrome *c* (12) the Loftfield–Bon nichsen method may have given too low values for several reasons The extraction of the tissue residues may have been unsatisfactory as cyto-chrome c in our control experiments has been recovered from the residues This may at least in part be due to the redistribution of cytochrome *c* on tissue elements as described by Beinert (2) Also the amount of dithionite may well have be n too small to ensure complete reduction The factors mention^d would both have contributed to too low values

Th present values for cytochrom^ *c* in human muscles are higher than such reported before but

Table IV *Content of cytochrome* c *in human skeletal muscle*

Determinations on 4 g of muscle Brackets indicate duplicate analyses

Diaphragm		Skeletal muscle	
Autopsy serial no	Cytochrome c (mg/100 g dry weight)	Autopsy serial no	Cytochrome c (mg/100 g dry weight)
351/67	24 05	⌊66/67	25 03
40/68	32 92	308 67	18 70
45/68	29 05	351/67	17 05
46 68	32 11	357/67	11 60
48 68	33 06	357/67	11 30
57/68	43 14	361/67	14 97
57/68	39 49	375/67	18 92
62/68	42 99	377/67	15 43
71/68	31 25	385/67	18 67
71/68	35 50	398/67	24 67
75/68	40 63	19/68	16 73
75/68	43 85	19/68	16 22
76/68	38 70	21/68	15 02
76/68	37 90	99/68	12 96
80/68	35 80	99/68	13 47
80 68	32 00	104 68	22 99
84/68	25 24	104 68	24 41
84/68	23 62	127/68	14 46
99/68	20 72	151/68	23 72
99/68	17 68	151/68	21 20
104/68	29 60	166/68	14 54
104/68	33 08	167/68	16 46
111/68	36 03	169/68	18 53
111/68	38 27	169/68	15 49
112/68	25 73	172/68	24 11
112/68	29 16	173 68	19 57
125/68	32 68	184/68	19 20
127/68	24 11	186 68	18 51
148/68	48 51	187/68	19 48
148/68	43 6⌊		
149/68	35 85		
149/68	28 64		
150/68	36 09		
151/68	31 36		
	33 31 ± 1 23		18 05 ± 0 74

Mean values gi en as mean ± s ε of mean

they may nevertheless be minimum figures if the alleged relationship of respiratory pigments to physical activity is true inasmuch as a majority of our subjects were older people suffering from chronic disease

Claims have been made by B utler (3 4) that in iron deficiency anemia respiratory enzymes like cytochrom *c* appear to becom⌣ depleted The part of th total iron content of the organism that belongs to cytochrom• *c* is nevertheless fairly small even with the data presented here

With higher absolute values biological differ

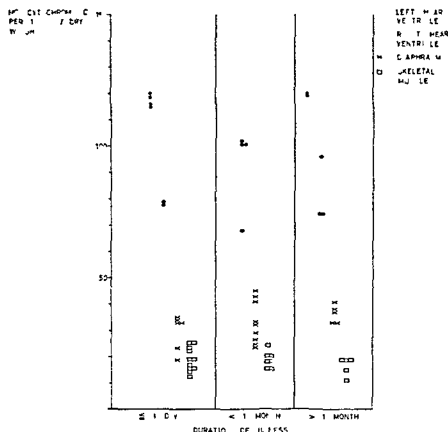


Fig 1 Relation between cytochrome *c* content and duration of illness

ences may be easier to establish but as our data show the range of values remains rather wide and a very great number of observations may be necessary to obtain significant differences in a material as heterogeneous as human subjects With the new method and data presented by us in a previous paper (1) and in the present one it might neverth less be worth while to make a venture into the tissue distribution of iron pig ments in conditions of obvious iron metabolism pathology (3 4 9)

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Acta m d scand Vol 185 pp 293–296 1969

ORGAN SPECIFIC ANTIBODIES IN IDIOPATHIC PANHYPOPITUITARISM PRIMARY THYROID AND ADRENAL INSUFFICIENCY

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Abst act Two hundred and forty-eight s ra from patients with idiopathic panhypopituitarism primary myxedema idiopathic Addison s disease as well as matched controls have been investigated for the presence of circulating organ specific antibodies

Antibody against the secretory cells of the anterior pituitary cou d not be demonstrated by means of the technique used In only one patient with pituitary failure was circulating antibody against thyroglobul n found and n ither microsomal thyroid antibody nor adrenal antibody could be detected in any case

Based on thes findings and a survey of the literature it is concluded that, in contrast to the findings in primary failure of the thyroid and the adrenals the functional and anatomical alterations in these endocrine glands in hypop tuitarism do not cause the formation of circulating organ ant bodies

Idiopathic panhypopituitarism (i p) i e pituitary insufficiency in which no cause can be demon strated and so called Sheehan s syndrome may closely imitate primary myxedema and although less frequently Addison s disease

Circulating antibodies against elements of the thyroid gland can be demonstrated in most sera from patients with primary myxedema (14 15 27 28) and it has been suggested that primary myxedema is identical with or a variant of Hashi moto s thyroiditis (4 8 10) The occurrence of thyroid antibodies in various diseases of the thyroid gland has been studied by several in vestigators (10 14 15 17 20 22 25 28) How ever only one study comparing the occurrence of thyroid antibodies in primary and secondary myxedema has been published (31)

A numb r of papers on the occurrence of cir culating antibody against the cytoplasm of adreno-cortical cells in sera from patients with different types of primary adrenal insufficiency have ap peared (1 2 6 7 23 24) but no information on the possible occurrence of adrenal antibodies in secondary adrenal insufficiency is available

Recently the theory has been advanced that i p may be an autoimmune disorder (12 19) and in 18 % of a group of women in the post partum period and in one case of so-called Sheehan s syndrome Engelberth and Jezkova demonstrated organspecific like activity against the adenohypo physis (9) However Goudie (13) in a preliminary study has so far been unable to confirm the existence of antibodies against the secretory cells of th anterior pituitary

The purpose of this paper is to present the re sults of an investigation on the problem of whether or not the functional and anatomical changes in the thyroid and adrenal glands as seen in pituitary insufficiency may be correlated to the existence of organ antibodies An attempt was made to demonstrate circulating antibodies against the secretory cells of the anterior pituitary in sera from patients with i p using an indirect immunofluorescence technique

MATERIAL

Sera from a total of 248 individuals were examined In 16 a d agnosis of i p was cons dered certain since no cause of the disorder could be demonstrated Five out of nine female patients were diagnos d as having Shee han s syndrome

All patients presented the classical symptoms and s gns of p tu tary insufficiency In all of them the excret on of pitu tary gonadotropins and corticosteroids (17 KGS) were low as was the level of se PBI (except in one case in whom iodine contamination was obviously pres nt) In most patients the diagnosis was supported by additional investigations such as stimulation tests with thyrotropic and adrenocorticotropic hormones, and measurement of plasma cortisol concentration

In addition sera from 60 patients with primary myxe dema and 48 pat ents w th idiopath c Addison s d seas were examined

Table I *Occurrence of antibodies against thyroid adrenal parietal cell salivary gland and anti nuclear factor in sera from patients with idiopathic panhypopituitarism and their respective control group (control group I)*

Cyto = Microsomal thyroid antibody demonstrated by the immunofluorescence technique Trc = Tanned red cell test for antithyroglobulin Ca_2 = Antibody against a colloid antigen different from thyroglobulin demonstrated by the immunofluorescence technique Par = Parietal-cell antibody demonstrated by the immunofluorescence technique Sal = Salivary gland antibody demonstrated by the immunofluorescence technique

			Idiopathic panhypopituitarism							Control group I						
			Thyroid							Thyroid						
No	Sex	Age	Cyto	Trc	Ca_2	Adrenal	Par	Sal	ANF	Cyto	Trc	Ca_2	Adrenal	Par	Sal	ANF
1	ɔ	51		–	–	–	–	–	–	–	–	–	–	–	–	–
2	o	67	–		–	–	–	–	–	–	–	–	–	–	+	+
3	o	30			–	–	–	+	–	–	–	–	–	–	–	–
4	o	47		–	–	–	–	–	–	+	–	–	–	+	+	–
5	o	70		–	–	–	–	–	–	–	–	–	–	–	–	–
6	o	33	–	–			–	–	–	–	25	–	–	–	–	–
7	ɔ	70	–	–	–	–	–	–	+	–	–	–	–	–	–	+
8		26	–		–	–	–	–	–	–	25	–	–	–	–	–
9		66		50		–	–	–	–	–	–	–	–	–	–	+
10		53			–	–	–	–	+	–	–	–	–	–	–	+
11		53	–		–	–		–	+	+	–	+	–	–	–	–
12		50	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13		5[illegible]		–	–	–	–	–	–	–	–	–	–	–	–	–
14		62			–		–	–	–	–	250	–	–	–	–	–
15		43	–		–	–	–	–	–		–	–	–	–	–	–
16		55			–	–	–	–	–		–	–	–	–	–	–

Finally an identical number of control persons matched according to sex and age to each of the groups mentioned was included in the study (control group I *16 persons control group II 60 persons control group* III 48 persons)

The results of the studies on the patients with primary myxedema and adrenal insufficiency have been published previously (14 –4)

METHODS

Microsomal thyroid antibody was demonstrated by an immunofluorescence technique as described by Holborrow et al (18) Thyroglobulin antibody was measured by means of thyroglobulin-sensitized sheep red cells from Burroughs Welcome & Co Ca 2 antibody was demonstrated according to Balfour et al (3) using the immunofluorescence technique on methanol fixed thyroid sections

Antibodies against parietal cells adrenal cortex and salivary gland were demonstrated by immunofluorescence techniques using unfixed sections of human gastric mucosa salivary gland and adrenals from guenon and man as previously described by Irvine (1) Bertram and Halberg (5) and Blizzard et al. (6)

Only sera in which thyroglobulin antibody was not present at all, or present in very low titres (< 50) were examined for the presence of the Ca 2 antibody because the thyroglobulin antibody may prevent the demonstration of the Ca 2 antibody

An attempt to demonstrate antibodies against the secretory cells of the anterior pituitary was made by means of an indirect immunofluorescence technique in the following way normal pituitaries from rabbit, monkey and man were used as antigens The human glands were surgically removed from patients with cancer of the *breast (Prof N Riskær) After removal the pituitaries* were immediately frozen and kept at –70 C until used 6 μ cryostate sections were incubated with sera for varying intervals (30–60 min) at varying temperatures (4–37 C) The slides were washed with barbiturate buffer (pH 7 0) (3) for a varying length of time (10 30 min) in an excess of buffer at varying temperatures (room temperature – 37 C) This procedure was followed by incubation with 10 μl fluorescein labelled antihuman globulin (horse origin Progressive Lab Maryland, USA) for 30 min at room temperature After an additional washing in buffered saline the sections were mounted in a drop of glycerine buffer and immediately studied under the fluorescence microscope (Reichert Zetopan)

RESULTS

Attempts to demonstrate circulating antibody against elements of the anterior hypophysis by means of the technique described were unsuccessful

The presence of other organ antibodies is listed in Tables I II and III Table I shows that thyroid antibodies very infrequently could be demonstrated in sera from patients with i p Serum from only one patient contained thyroglobulin antibody in a low titre In the corresponding control group

Table II *Occurrence of thyroid antibodies in sera from patients with idiopathic panhypopituitarism primary myxedema and the corresponding control groups*

	No	Cyto	Trc	Ca 2	One or more antibodies
Idiopathic panhypopituitarism	16	0	1 (6)	0	1 (6)
Control group I	16	2 (12)	3 (18)	1 (6)	5 (31)
Primary myxedema	60	39 (65)	46 (79%)	17/35 (48)	57 (95)
Control group II	60	6 (10%)	9 (15)	4/35 (11)	20 (33%)

one or more thyroid antibodies could be demonstrated in sera from five persons The results indicate that thyroid antibodies occur less frequently in sera from patients with secondary myxedema than in control sera (Chi test with Yates s correction $0.01 < p < 0.02$)

Adrenal antibody could be demonstrated neither in sera from patients with i p nor in sera from control persons (control group I) while antibodies against parietal cells and salivary glands as well as antinuclear factor were found with the same frequency in both groups

Table II shows the occurrence of thyroid antibodies in primary and secondary myxedema These antibodies were found in only one patient with pituitary myxedema On the other hand one or more thyroid antibodies could be demonstrated in sera from 95 of the patients with primary myxedema The fact that thyroid antibodies were found with the same frequency in the two control groups (control group II and III) indicates that the difference between the findings in primary and secondary myxedema is a real one and not merely an expression of a different composition of the two materials as regards sex and age

Table III shows that adrenal antibody could b demonstrated in 66° of the patients with primary adrenal insufficiency—i e patients with idiopathic Addison s disease

Table III *Occurrence of adrenal antibody in sera from patients with idiopathic panhypopituitarism idiopathic Addison s disease and the corresponding control groups*

	No	Adrenal antibody
Idiopathic panhypopituitarism	16	0
Control group I	16	0
Idiopathic Addison s disease	48	31 (66)
Control group III	48	0

DISCUSSION

Since it is questionable whether small necroses of the adenohypophysis resulting from post partum hemorrhage may be solely responsible for the development of panhypopituitarism cases of so-called Sheehan s syndrome are included in this study

Patho anatomical changes in the anterior lobe of the pituitary justifying the term chronic lymphocytic hypophysitis have been reported in very few cases (12 19 29 33) Only in the two cases reported by Goudie and Pinkerton (12) and Hume and Roberts (19) are the changes so severe and extensive that a pathogenetic importance of the lesions may reasonably be suspected and an autoimmune mechanism be suggested No such cases were included in the big series reported by Sheehan and Summer (30) and they may possibly be quite exceptional occurrences Lymphocytic infiltration in the pituitary is not a characteristic finding in healed post partum lesions The round cell infiltration seen in pituitaries adjacent to giant cell granulomas is of an entirely different type (26 30)

The failure to demonstrate pituitary antibodies in this study may be explained either by an insufficient technique or by the fact that the disorder in which the occurrence of an antihypophyseal antibody may be anticipated—i e chronic lymphocytic hypophysitis—is so exceptional that it may not be included in the material presented

The infrequent demonstration of thyroid antibodies in sera from patients with i p is in agreement with the study of Valloton et al on patients with secondary hypothyroidism (31) It seems justified to postulate that unlike primary myxedema, th anatomical and functional changes of the thyroid in pituitary insufficiency do not cause the appearance of circulating antibodies This point deserves considerable interest as it may

prove helpful in the often difficult differential diagnosis between primary and secondary myxedema

When post mortem examinations were carried out changes in the thyroid gland essentially indistinguishable from those present in Hashimoto s thyroiditis and primary myxedema were found in 57% of the cases described by Sheehan and Summer (30) a figure which is in accordance with the frequency with which asymptomatic thyroiditis is found in elderly women in larger autopsy materials (11 16 32) Furthermore it is well established that one or more circulating thyroid antibodies may be found in about 30% of apparently healthy persons above the age of 50 The findings in i p may serve as an example of thyroiditis without concomitant occurrence of antibodies Whether the inactivity atrophy of the thyroid causes quantitative and/or qualitative changes of the thyroid antigens so as to make the formation of thyroid antibodies impossible remains to be elucidated

The adrenal cortex in i p shows considerable atrophy whereas scattered round cell infiltration has been reported only in one case (30) Consequently the failure to demonstrate adrenal antibody might be anticipated

The functional and anatomical changes of the adrenals in secondary hypoadrenalism do not give rise to the formation of adrenal antibodies

CONCLUSION

On the basis of these findings the following conclusions seem justified

With th technique employed no antibodies against the anterior lobe of the hypophysis can be demonstrated in sera from patients with idiopathic panhypopituitarism

Contrary to the findings in primary myxedema and idiopathic Addison s disease the functional and anatomical alterations of the thyroid and the adrenals in pituitary failure do not cause the formation of circulating thyroid or adrenal antibodies This finding is of importance in the differential diagnosis between primary and secondary failure of the thyroid and the adrenals

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LIDOCAINE AS AN ANTIARRHYTHMIC AGENT

Experience in 68 patients

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Abstract Lidocaine has been used in the treatment of 93 episodes of arrhythmias in 68 patients in a coronary care unit Forty nine of the patients had acute myocardial infarction A booster dose of 25 50 or 100 mg was given initially When effective this injection was followed by intra enous infusion of /₂–4 mg/min when a prolonged effect was desired In this series lidocaine proved effective in the treatment of ventricular extrasystoles and tachy cardia and facilitated cardioversion in some patients with ventricular tachycardia or fibrillation whereas few cases of supraventricular arrhythmias could be controlled in this way Side-effects especially hypotension were rare and as a rule they subsided after brief discontinuation of the drug This was true also in two cases of supra ventricular tachycardia following lidocaine treatment How ever this drug might be incriminated as contributing to the death of two patients with myocardial infarction. Over quinidine and procainamide lidocaine has the ad antages of a rapid onset of a tion and rapid elimination Besides deleterious circulatory side-effects seem to be very rare

Lidocaine was synthesized in 1943 by Lofgren (13) and subsequently extensively used as a local anesthetic agent The successful use of lidocaine in association with AC shock in a case of ven tricular fibrillation arising during cardiac cathe terization was reported in 1950 (18) The efficacy of lidocaine in the management of cardiac ar rhythmias during surgery especially cardiac sur gery has been demonstrated by several authors (8 23)

In recent years evidence has accumulated that cardiac arrhythmias complicating acute myocardial infarction are much more common than was previously thought (3 25) In the treatment of these arrhythmias especially ventricular arrhyth mias lidocaine has turned out to be useful as revealed by several recent publications (6 9 12)

PHARMACODYNAMIC EXPERIMENTAL AND CLINICAL BACKGROUND FOR THE USE OF LIDOCAINE AS AN ANTIARRHYTHMIC AGENT

Pharmacodynamics of lidocaine

Structurally lidocaine is very similar to procaine (Fig 1) It is highly soluble stable and non irritating locally The onset of action of lidocaine occurs rapidly due to its fast diffusion and penetration of cell membranes

Lidocaine is metabolized in the liver by de-ethylizing and amide splitting enzymes (26) It is excreted in the urine as free and conju ated phenols Less than 10% is excreted unchanged (5)

Lidocaine can be determined in blood and tissue by colorimetry or gas chromatography (22) Therapeutic blood levels as assessed by the latter method are supposed by Gianelly to be 2–5 μg/ml (6) whereas Jewitt found that 1 5–2.5 μg ml was associated with a satisfactory effect (9) This level was achieved immediately after a booster dose whereas / 1 / hours might elapse when infusion of 1–2 mg/min was given without a booster dose In animal experiments it was shown by colorimetry that after a single intravenous injection of lidocaine the concentrations in the central nervous system in the heart and in fat tissue were significantly higher than in the blood A rapid fall was seen in all tissues except fat tissue where the concentration was unchanged after 30 min, pointing to a cumulation of the drug Ten minutes after the injection only half of the injected dose could be reco ered from the tissues (21)

Mechanism of action

The mechanism of action of lidocaine does not differ from that of other local anesthetics Thus it interferes with the fun tion of all structures in which generation conduction or transmission of impulses occur such as the *central nervous system the autonomic ganglia the myo*-neural junctions, and all sorts of muscle fiber (15) Local anesthetic agents act on the cell membranes of these structures bringing about a reduction of their permeability to sodium and potassium ions The e act mechanism of this action is unknown at present.

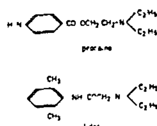


Fig 1 Structural formula of procaine and lidocaine

Thus the antiarrhythmic action of lidocaine is similar to that of procaine It depresses the excitability of the myocardial cell slows conduction velocity and prolongs the refractory period The function of the sino atrial node and myocardial contractility should not be affected by the usual therapeutic dosages (5)

Dosage and administration

When used as an antiarrhythmic agent lidocaine is always given intravenously Usually a booster dose of 25 50 or 100 mg (1-? mg kg) is injected initially If this is ineffective it can be repeated after 10 ?0 min due to the rapid dissipation of this agent Usually a prolonged effect is desired and so an intravenous infusion giving 1–4 mg/min is started at the same time (6 9 12, 19)

Influence of lidocaine on circulation

The influence of lidocaine on the hemodynamic state has been investigated in animal experiments (1 10) In man hemodynamic examinations have been carried out in patients undergoing cardiac (7) or other kinds of surgery (11) in patients with acute myocardial infarction (9 ?0) and in patients with other kinds of heart diseases (16) The results of these investigations indicate that lidocaine has no significant circulatory side-effects when given in therapeutic dosages

Side-effects

These are mainly due to the effect of lidocaine on the central nervous system and the myocardium As pointed out by Jewitt (9) the majority of possible side-effects are rare after a single intravenous injection but they are seen now and then in patients treated with lidocaine infusion The only side-effect which has been reported rather often is drowsiness, which may appear even at low dosages Overdosage of lidocaine may result in conduction disturbances or bradycardia and signs of cortical irritation such as muscular twitching and fasciculations which may progress to convulsive seizures (4 5) In rare cases this may happen even with therapeutic" infusion rates (17) Psychotic reactions with apprehension and disorientation have been reported in a few cases (5) Ventricular tachycardia occurred on two occasions in the same patient at the end of an injection of 50 mg of lidocaine (14) When given in big dosages lidocaine may provoke cardiovascular collapse due to depression of the central nervous system and the myocardium.

Table I *Distribution as to diagnosis and sex of 68 patients treated with lidocaine*

Diagnosis	Total	Men	Women
Acute myocardial infarction	49	36	13
Coronary artery disease	8	7	1
Miscellaneous	11	8	3
Total	68	51	17

AUTHORS MATERIAL

Subjects

In the Unit for Intensive Coronary Care (in Medical Department B University Hospital Copenhagen) we have used lidocaine as an antiarrhythmic agent since March 1956 During this period we have treated 68 patients (51 men and 17 women) aged 36 78 years. Table I shows the distribution as to sex and diagnosis Forty nine patients had acute myocardial infarction Table II lists the types and numbers of arrhythmias treated with lidocaine

Principles of treatment and indications

Lidocaine has been given only intravenously A booster dose of 25 50 or 100 mg was given initially However three patients received 150 mg. When effective the booster dose was usually followed by a continuous infusion of lidocaine in dosages of $^1/_2$–4 mg/min

This kind of treatment has been tried in a number of patients with supraventricular arrhythmias, but the principal indications have been ventricular arrhythmias complicating acute myocardial infarction According to Lown et al (12) our indications for treating ventricular extrasystoles in such patients have been 1) A frequency exceeding 5–10 ?) multiform extrasystoles 3) extrasystoles in salvos and 4) extrasystoles interrupting the T wave

Patients with ventricular tachycardia have been given a booster dose of 50–100 mg of lidocaine initially If this treatment failed to control the arrhythmia, it was followed by a DC shock In ventricular fibrillation lidocaine in the same dosages was often given immediately before a DC shock After conversion of ventricular tachycardia

Table II *Intravenous lidocaine*

Figures in brackets indicate number of patients with acute myocardial infarction

Type of arrhythmia	No of episodes	Effect +	Effect −
Supraventricular ectopic beats	6	3 (3)	3 (3)
Supraventricular tachycardia	3	[illegible]	1 (1)
Atrial flutter	4	0	4 (3)
Atrial fibrillation	8	1 (1)	7 (3)
Ventricular ectopic beats	31	24 ( )	7 (3)
Ventricular tachycardia	18	7 (?)	11 (7)

and fibrillation lidocaine infusion has usually been given to prevent another attack.

Lidocaine infusion has been continued for 24 or 48 hours in most patients In some cases this treatment had to be carried on for a longer period the maximum bein, five days. During the infusion and after the injection of a booster dose we have controlled heart rate P–R and QRS intervals frequency of extrasystoles and blood pressure

Total atrioventricular block and bradycardia have been regarded as contraindications for treatment with lidocaine

RESULTS

In this series of 68 patients 93 episodes of arrhythmias have been treated with lidocaine The types of arrhythmia and the number of episodes in which lidocaine had a beneficial effect will be seen from Tables II and III

Most of the supraventricular arrhythmias in which treatment with lidocaine was tried could not be controlled by this agent

In 24 out of 31 cases of ventricular premature contractions which were treated with lidocaine total elimination or a significant reduction of frequency was achieved Fig 2 demonstrates the elimination of coupled ventricular extrasystoles two min after an injection of 25 mg of lidocaine

In seven cases a ventricular tachycardia could be controlled with lidocaine alone The first strip of Fig 3 shows the initiation of a ventricular tachycardia by an extrasystole Seventy two sec after the termination of an injection of 50 mg of lidocaine a stable sinus rhythm was restored as demonstrated by the third strip In this case three DC shocks had been given without effect before treatment with lidocaine was tried In five cases lidocaine alone could not restore sinus rhythm which was achieved easily by a subsequent DC shock

In ventricular fibrillation lidocaine has not been used alone except in a very few cases in which defibrillation for some reason could not be carried out immediately Lidocaine alone has never had

Table III *Lidocaine and DC shock*

Figures in brackets indicate number of patients with acute myocardial infarction

Type of arrhythmia	No of episodes	Effect +	Effect –
Ventricular tachycardia	11	5 ( )	6 (5)
Ventricular fibrillation	23	15 (14)	8 (3)

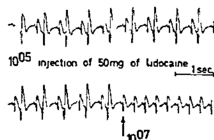


Fig 2 Bipolar chest lead from a 78 year-old man with acute myocardial infarction complicated by coupled extra systoles (indicated by dots)

effect in any of these cases Fifteen cases were treated with a booster dose of lidocaine followed within 2 min by a DC shock In some of these cases several preceding DC shocks had failed to restore sinus rhythm

Side effects

Side-effects of lidocaine treatment were rare and as a rule reversible Especially it should be noted that significant hypotension was seen only in one case which will be commented on below

Drowsiness was seen in several patients however this is not undesirable in patients with acute myocardial infarction and does not indicate intoxication One patient was disturbed by a feeling of difficulty in breathing Slight prolongation of P–R intervals was seen in three patients one of whom had also a broad QRS All the above mentioned symptoms disappeared after discontinuation of the treatment

In two patients treated with lidocaine infusion a psychotic reaction with anxiety and confusion developed during the treatment One patient at that time received 4 mg/min the other inadvertently 8 mg/min The latter said afterwards that he had had double vision and difficulty in speaking before the psychotic reaction In the course of the 24 hours within which this took place he received a total of 7650 mg without the occurrence of hypotension or electrocardiographic signs of overdosage In both patients the symptoms disappeared after a brief discontinuation of the treatment

One patient with a large anterior wall infarction developed circulatory arrest eight days after the onset of symptoms The ECG at that time showed a supraventricular tachycardia DC shock

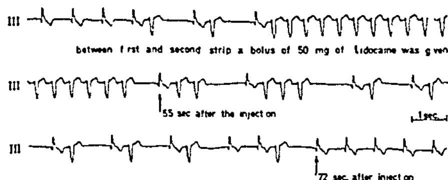


Fig. 3. Third standard lead from a 49-year-old man with acute myocardial infarction complicated by attacks of ventricular tachycardia. Stable sinus rhythm was obtained 72 sec after injection of lidocaine.

and antazoline did not restore sinus rhythm. Then 150 mg of lidocaine was injected and immediately brought about asystole which was resistant to all further treatment.

In two patients supraventricular tachycardias occurred following treatment. One patient had pre-excitation and numerous ventricular extrasystoles up to 50%. A few minutes after an injection of 50 mg he had a sinus tachycardia at 160/min. It disappeared after 20 min. The heart rate before and after lidocaine is seen in Fig. 4.

The other patient had acute myocardial infarction complicated by attacks of ventricular fibrillation and tachycardia. For this reason he had been treated with lidocaine infusion 2 mg/min for 12 hours. At that time he developed a supraventricular tachycardia which disappeared 20 min after discontinuation of the drug. On another occasion he had ventricular fibrillation immediately after injection of 50 mg of lidocaine. Sinus rhythm was easily restored with a DC shock.

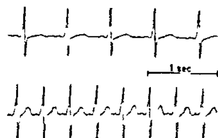


Fig. 4. Bipolar chest lead from a 47-year-old man with pre-excitation. Heart rate before and 5 min after injection of 50 mg of lidocaine.

A 78-year-old man with acute myocardial infarction received lidocaine infusion for 84 hours because of coupled ventricular extrasystoles. The treatment had a good effect on the extrasystoles but suddenly convulsive seizures and cardiovascular collapse developed. Lidocaine was stopped but the patient died 2 ¹/₂ hours later. The autopsy showed a large infarction of septum and the anterior wall and severe coronary sclerosis with total occlusion of the anterior descending branch.

DISCUSSION

In keeping with the results of other authors we have found that lidocaine is of little value in the treatment of supraventricular arrhythmias. In these cases the well established treatment with digitalis preparations, quinidine or DC shock should be preferred (9, 19).

Ventricular tachycardia could be reversed with lidocaine alone in some cases, whereas in others a DC shock was required. In a number of cases we have been impressed by the fact that a booster dose of lidocaine facilitated cardioversion when given to patients with ventricular tachycardia or fibrillation in whom one or more preceding DC shocks had been ineffective.

In animal experiments it has been demonstrated that there is a vulnerable period for ventricular tachycardia following myocardial infarction (24). Clinical observations in patients with acute myocardial infarction indicate that premature ventricular contractions in some cases are the prodromata of ventricular tachycardia or fibrillation (12). Consequently many authors recommend

vigorous treatment of ventricular extrasystoles in these patients as they think that this will greatly reduce the frequency of such serious arrhythmias This problem still requires further evaluation as it is not known how many patients with myocardial infarction and ventricular extrasystoles do not develop serious arrhythmias

It is very difficult to evaluate the effect of lidocaine on the frequency of ventricular extrasystoles in some cases in which a total elimination is not achieved immediately after initiation of treatment as the number of extrasystoles varies very much from time to time Another factor contributing to these difficulties is the transient nature of extrasystoles in myocardial infarction Counting of the number of extrasystoles per 100 beats every 15 min at the beginning and every 30 min later on as we have done in this series is not sufficient

Significant side-effects were rare in this series and in most cases reversible Two instances of irreversible side effects leading to the death of the patients occurred Convulsive seizures and cardiovascular collapse developed in one patient during lidocaine infusion and asystole in another after injection of 150 mg preceded by injection of antazoline In both these cases it might be suspected that lidocaine contributed to the death of the patients In two cases supraventricular tachycardia occurred following lidocaine treatment To our knowledge this has not been reported earlier in the literature

In the case of serious side-effects lidocaine should of course be discontinued As a rule the symptoms will disappear in 15–30 min Hypotension should be treated by elevation of the legs and if necessary with a pressure amine of norepinephrine type as it has been shown that lidocaine potentiates the blood pressure responses of this amine (2) Bradycardia should be treated with atropine and convulsions with barbiturates

In our opinion total atrio-ventricular block and bradycardia should be regarded as contraindications for lidocaine treatment unless effective pacing can be performed In the presence of other kinds of disturbances of conduction or impulse formation lidocaine should as a rule not be used and only with extreme caution It is contraindicated in severe liver damage

Accurate control of infusion dose over long periods is a crucial point Side-effects due to excess dosage could be limited if a constant rate infusion pump is used instead of standard infusion sets

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DIABETIC AMYOTROPHY—A WELL DEFINED SYNDROME?

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Abstract Seventeen case reports from diabetics with muscular weakness and wasting of the extremities are reviewed briefly Symptoms signs and neurophysiologi al findings are discu sed in the light of earlier reports, in cluding histological studies The clinical picture in periph eral diabetic neuropathy is variable Sometimes sensory symptoms dominate and sometimes motor symptoms pre vail. However neuropathological and neurophysiological findings seem to be identical It is concluded that there is no proof of the existence of a pure motor neuropathy as a well-defined syndrome in diabetes mellitus as su gested by Garland

In 1953 Garland and Taverner (4) described a neurological syndrome in diabetics consisting of asymmetrical pain weakness muscle wasting and areflexia in the legs without objective sensory disturbance They called the syndrome diabetic myelopathy but Garland (5 6 7) in later publica tions has preferred the term diabetic amyotrophy as clinical or histological evidence of a spinal lesion is often lacking Several authors have since contributed to the discussion of this clinical picture but it is still not clear whether a purely motor syndrome can be construed with sufficient clarity to merit a special designation such as d m or d a

During the last six years I have been concerned with neurophysiological studies in patients with diabetes During these years I have observed the neurologic symptoms and findings in the ca 1200 diabetics under regular control in the Diabetes Clinic of the Department of Medicine and in the patients with neuropathy and diabetes whom I have seen in other departments of the Aarhus Kommunehospital

The most conspicuous feature in the syndrome described by Garland is paresis and muscle atrophy In the following a short description will be given of the 17 cases where these two find ings were present among a group of 214 diabetics selected for neurophysiological studies Eleven of these 214 patients were from the Department of Neurology and two from the Department of Ophthalmology The rest were from the Depart ment of Medicine or the Diabetes Clinic On the basis of these case histories and the experien e of others a discussion of whether there is reason to retain the idea of a special motor form of diabetic neuropathy will be presented

CASE REPORTS

Abbrev ations

VPT vibratory perception threshold as measured with a Biothesiometer (Bio-medical Instrument Co Chagrin Falls Ohio USA) The result is expressed in volts (0 50) On the great toe the vibratory perception threshold is about 5-10 volts in normal young persons It rises gradually with a e at age 60 it is u ually between 10 and 30 volts

MCV motor conduction velocity was defined by the d stance between two stimulation points on a peripheral ner e divided by the d fference in the latency time to the muscle response and expressed in meters per second Normal ran e in the peroneal nerve is from 43-59 m sec in young peoples and from 41 55 m sec at older age

EMG electromyography performed by insertion of three coaxial needles in the muscle In the present work pathological findings are with few except ons described as loss of motor units during maximal voluntary effort

Case 1

A 27 year-old man who had had diabete for seven years He had been treated with insul n but poorly controlled On year pr or to admis ion he complained of lancinating pains in both le s after diabetic coma At that time he had lost 23 kg in we ght The blood sugar level was about 150 mg/100 ml on admission Ophthalmoscopy showed d abetic retinopathy Neurologic examination re ealed

muscular *atrophy* and marked a dysesthesia and hypalgesia in MCV in the peroneal nerve was the extensor dig brevis and rectus owed severe loss of motor units VPT a volt

insulin dose was increased and his weight rea d 27 kg over the following 2 ¼ years was prepared His muscular strength increased but h was not able to perform normal work the ja de rea ed and hypalgesia dis rmal de p reflexes returned in the upper weak patellar reflexes in the legs MCV VPT howed only questionable improvement

woman with diabetes of 14 years duration. en tr ated with insulin but poorly controlled, precoma shortly before admission There e symptom On admission the blood about 175 mg 100 ml and ophthalmoscopy ive diabetic retinopathy Neurologic ex and mild *reduction in strength* and *atrophy* p muscles weak patellar reflexes and eflexe hypesthesia and hypal esia in peroneal nerve) was 38 m/sec EMG brevis muscle howed slight reduction of VPT measured on the great toes wa 13

A 44 year-old man with diabetes for 35 years He had always had mild diabete and only during the last five year had he needed a small amount of insulin to a hieve good regulation The blood sugar was normal on admission There was diabetic retinopathy An attempt was made to discontinue insulin therapy but the blood sugar level rose gradually over a two month period to 400 mg/100 ml At this point a right sided *drop foot* developed There was *atrophy* of both extensor dig brevis mu cles brisk deep reflexes hypesthesia and hypalgesia in the ri ht foot MCV in the right peroneal nerve 47 m/sec in the le peroneal nerve 38 m sec EMG of both extensor di evis muscles and both tibialis anterior muscles reve evere reduction in motor units VPT on the right grea e was 44 volts on the left great toe 34 volts

The atient was again placed on insulin therapy and the pero eal paresis improved appreciably in the course of a few days After seven months the paresis had disappeared ompletely

Case 4

A 38 year-old woman with a duration of diabetes of 27 years *Insulin treatment was difficult to regulate resulting* in severe fluctuation in blood sugar values There was severe proliferative retinopathy Three years before admission the patient had an apoplectic insult with paralysis of the right arm In the legs there was *reduced strength* in the toe extensor on both sides There was *at ophy* of the left quadricep and both ext dig br muscles The right patellar reflex was weak but otherwise there was deep areflexia Hypesthe ia and hypalgesia distal to the knees was also found MCV in the peroneal 33 m/sec EMG of the extensor dig brevis mus severe loss of motor units VPT (great toe) w

The patient died eight months later sufficiency

Case 5

A 52 year-old man who had h year and who had been treat three years before admissio of low back pain radiati down to the lateral side spontaneously On a weeks of similar p by right-sided dr fore admission Th 100 ml There amination revealed with *atrophy* of the ri ht reflexes were retained but Ach Laseque s sign wa negative or b h reduced sensitivity to pin prick and tou side of the lower legs and foot MCV wa in both peroneal nerves EMG of the right tibial and ext dig brevis muscle showed a severe loss of units VPT on the great toes was 20 volts

The right-sided drop foot was unchanged 4 ½ years later

Case 6

A 56-year-old man with recently diagnosed diabetes On admission the patient complained that for the last two years he had had lancinating pains in both legs which were later followed by a subjective sensation of reduced muscular strength and difficulty in walking. During the last 5 to 10 years there had been periods of thirst and he had lost 15 kg in weight The blood sugar level was around 300 mg/100 ml on admission and there was diab tic retinopathy A neurologic examination showed slightly *reduced st ength* in the quadriceps muscles *atr phy* of both extensor dig brevis muscles and moderate reduction in strength in the dorsiflexors of the foot There were fasci ulations in both lower l gs Weak patellar reflexes missing Achilles reflexes and hypesthesia algesia of both feet were also found MCV in the right peroneal nerve 31 m/sec in the left peroneal nerve it was unmeasurable EMG from both extensor dig brevis muscles showed a severe loss of motor units. The VPT was >50 volts on both great toes

The patient was placed on a diet and seen a ain four months later He had gained three kg and the blood su ar level was normal The subjective complaints and the objective findings were unchanged except that the patellar reflexes were now also missing MCV EMG and VPT were un hanged

The patient died half a year later of a bleeding stomach ulcer

Case 7

A 62 year-old woman who had had diabete for five months She had been treated with chlorpropamide Shortly after the institution of treatment the patient had pains in

the left gluteal region and left leg, which were most pronounced at night accompanied by difficulty in controlling the left foot. The pains disappeared spontaneously in the course of three months On admission the blood sugar level was about 150 mg/100 ml. There was no retinopathy A neurologic examination revealed *atrophy* and *weakness* of both quadriceps and of the left tibialis anterior muscles There was deep areflexia in both lower extremities and reduced sensation for pin prick and touch on the left foot. MCV in the right peroneal nerve was 43 m/sec and unmeasurable on the left side EMG showed a se ere loss of motor units in the left rectus femoris left tibialis anterior and both extensor dig brevis muscles VPT on the great toes was >50 volts

Case 8

A 56-year-old man in whom diabetes mellitus had been diagnosed two months before admission and thereafter treated with chlorpropamide Prior to this the patient had had thirst for several months and a weight loss of ten kg, together with constant pains most pronounced at night in the left lower back, gluteal and femoral regions He had difficulty climbing stairs On admission the blood sugar level wa approx. 150 mg/100 ml There was no retinopathy Neurologic examination revealed *atrophy* of the left gluteus medius thigh and lower leg and severe *eakness* of the left quadriceps. The left patellar reflex could not be elicited the other deep reflexes in the lower extremities were weak. There was hypesthesia algesia of both lower legs MCV in the left peroneal nerve was 43 m/sec EMG from the left rectus femoris muscle showed a severe loss of motor units and from the left extensor dig. brevis muscle interference with numerous denervation potentials was recorded EMG from the right rectus femoris muscle showed nothing abnormal. VPT (great toes) >50 volts

Th patient was seen on follow up eight months later The blood sugar level was around 1 0 mg/100 ml The pains and paresis of the left leg had decreased but a similar picture had developed on the right side Neurologic examination revealed severe paresis of the right quadriceps and tibiali anterior muscles and mild paresis of the cor re pordin mu cle on the left side There wa deep areflexia in the leg and hypesthe a alge ia in both lower l gs. *EMG from the l ft re tus femoris muscle* now showed more motor units than previou ly There wa now however severe loss on the right side

Case 9

A 67 year-old woman with diabete for four years. She had b n treated with tolbutamide but poorly regulated Durin the months precedin admission the patient had lost we ght and she had developed pa n in both gluteal region and lower legs and gradually paresis of the l ft foot. On admission the blood sugar level was approx 00 m 100 ml there was no retinopathy Neurologic examination revealed mild, diffuse *atrophy* of the mu cl s of th lower extremi ies, mild *weakness* of the right quad i eps and tibialis anterior muscles, severe paresis of th dorsiflexors of the left foot The right patellar and left Achille reflexes were la king. Sensation wa normal. MCV in the right peroneal nerve 36 m sec in the l ft peroneal nerve 30 m/sec EMG from both rectus femoris and extensores dig. brevis muscles showed a moderate to severe loss of motor units VPT on the great toes was >50 volts

Insulin therapy was started and a normal blood sugar level achieved. The pain and paresis of the lower ex tremities, however became worse and hypesthesia of the lower legs gradually developed. The patient was seen one year later at a geriatric hospital. She was dement, immobile and there was atrophy and contractures of the upper as well as the lower extremities. She died shortly afterwards in that condition Autopsy showed severe renal papillary necrosis There was no macroscopic evidence of heart or brain damage

Case 10

A 51 year-old woman with a duration of diabetes of four months Treatment consisted of insulin She was admitted because of difficulty in walking. On admission the blood sugar level was about 200 mg/100 ml There was retinopathy Neurologic examination revealed *atrophy* of both gluteal regions thighs and lower legs There was severe *weakness* of movements in the hips and knees. Both patellar reflexes and the right Achilles reflex were la king Sensation was normal

The dose of insulin was increased and the patient gained weight the blood sugar level became normal. On re admission nine months later diffuse weakness was found in both legs with paralysis of the dorsiflexors of both feet and deep areflexia. There wa hypesthesia algesia of both feet. MCV could not be mea ured in the peroneal nerves EMG showed severe loss of motor units in both extensor dig brevis muscles and the left tibialis anterior muscle EMG from the left rectus femoris muscle showed interference with many polyphasic potentials. VPT on the great toes was 70 volts

On later control admissions up to five year after the first admission the neurologic findings were essentially unchanged MCV EMG and VPT remained unchan ed too

Case 11

A 57 year-old woman w th recently d agnosed d ab es mellitus The patient had been sufferin from thirst for half a year and had lost 11 kg. One month before admi sion she suddenly developed *paresis* of the right foot On admission the blood sugar level was approx. 250 m 100 ml there was no retinopathy Neurologic examina tion revealed se ere right sided peroneal paresis, but other wise no abnormalities MCV in both peroneal nerves wa 45 m sec EMG from the r ght extensor d g. bre i muscle showed a severe loss of motor units. VPT (great toes) 40 volts

The patient was treated with tolbutamide the blood sugar le el became nearly normal and the peroneal pare is almost completely disappeared in the course of thre months. Thereafter the patient neglected both control and treatm nt and was first seen again four years later when she redeveloped an acute right-sid d *pe oneal pa esis* There was mild *atrophy* of the left and marked *at ophy* of the r ght extensor di bre is muscl weak patellar reflexes and absent Achilles reflexes. Sensat on was normal

with the exception of dysesthesia of the first toe inter stitium on the right foot. MCV in the right peroneal nerve was 33 m/sec as against 47 m/sec on the left side EMG in both extensor dig brevis muscles and the right tibialis anterior muscle showed severe loss of motor units *EMG from the right rectus femoris muscle revealed no* abnormalities VPT was >50 volts on both great toes

Treatment with tolbutamide was reinstituted and the paresis again disappeared in the course of three months MCV EMG and VPT changed only slightly during the *following year*

Case 12

A 61 year-old woman with diabetes of six years dura tion Diet was the only treatment given during the first *five years One year before admission instability developed* in the right leg Because of poor diabetes status and a weight loss of 25 kg insulin therapy was started Shortly thereafter weakness of the left leg and pain in both thighs developed On admission the blood sugar level was normal there was diabetic retinopathy Neurologic examination revealed marked *atrophy* and *weakness* of both quadriceps muscles deep areflexia and hypesthesia algesia in the lower legs EMG from the quadriceps muscle showed a severe loss of motor units and from the tibial anterior muscle a mild loss of motor units

The patient was seen in the diabetes clinic one year later she had discontinued insulin and again had ap preciable glucosuria The neurological symptoms and signs were unchanged however there was now paresis of the left foot s dorsiflexors in addition MCV in the left peroneal nerve was 40 m/sec EMG from the left extensor dig brevis muscle showed a severe loss of motor units VPT on the great toes was 24 volts

Case 13

A 70 year-old woman with diabetes for ten years She had been treated with insulin during the first four years There had been a period of lancinatin pains in the left leg and weakness on extension of the left knee at the ime of the appearance of diabetes Half a year before examination she had lancinatin pains in the entire right leg and weakness on extension of the right knee Insulin treatment was again started At the time of examination the blood sugar level was about 170 mg/100 ml There was diabeti retinopathy Neurologic examination revealed *slight atrophy of both quadriceps mild weakness on exten* sion of the right knee and dorsiflexion of the right foot and toes There was deep areflexia in the legs reduced sensation for pin prick and touch in both feet MCV in the right peroneal nerve was 40 m/sec EMG from the right rectus femoris muscle showed mild loss of motor units from the right tibialis anterior muscle interference denervation potentials from the right extensor dig brevis muscle severe loss of motor units VPT was >50 volts on both great toes

The patient was seen eight months later The blood sugar level was normal There was weakness of both quadriceps muscles and both tibialis anterior muscles and hypesthesia of both lower legs MCV in the right peroneal nerve was 30 m/sec EMG from the right rectus femoris muscle showed mild loss of motor units from the right tibialis anterior muscle and extensor dig brevis muscle severe loss of motor units VPT was unchan ed

Case 14

A 63 year old woman who had had diabetes for eight years The patient neglected control and treatment during the first 7 / years and only during the last / year before admission was she treated with chlorpropamide During the year before admission the patient had lancinat ing pains in the thighs and trouble in walking On ad mission the blood sugar level was normal There was retinopathy Neurologic examination revealed mark d *atrophy* and *paresis* of both quadriceps muscles deep areflexia in the legs hypesthesia algesia from the axillae downwards MCV in the peroneal nerve was 4? m/sec EMG of the quadriceps and extensor dig brevis muscles showed slight loss of motor units VPT on the right great toe was 19 on the left great toe >50 volts

The patient was seen on several occasions during the following two years during which time a gradual sub *jective and objective improvement in strength in the lower* extremities was seen The pains disappeared and sensa tion was reported as normal Deep areflexia in the le s persisted MCV EMG and VPT remained essentially unchanged

Case 15

A 50 year-old man with diabetes for 24 years who had been treated with insulin with frequent episodes of insulin shock One year before admission progressive weakness *in both arms began On admission the blood sugar level* was found to be quite variable There was retinopathy Neurologic examination revealed diffuse *atrophy* and *weakness* of the musculature of the shoulder girdle and upper extremities There were fasciculations in both arms *and legs The biceps reflexes could be elicited* but there was otherwise deep areflexia Sensation was normal MCV in the ulnar nerve 57 m/sec in the peroneal nerve 41 m/sec EMG of the deltoid muscles and adductor pollucis showed a severe loss of motor units and fasciculation potentials EMG of the quadriceps and extensor dig brevis muscles revealed nothing abnormal VPT on the index finger was 10 volts on the great toes 23 volts

On readmission / year later progression of the paresis in the upper extremities was noted There was no involve ment of the cranial nerves EMG was unchan ed The patient died at home of pneumonia two years after the first admission

Case 16

A 63 year old woman with a duration of diabetes of 15 years Treated with insulin During the last three years a subjective weakness in the upper extremities was noted On admission the blood sugar level was about 230 mg/100 ml There was retinopathy Neurologic examination revealed mild *weakness* on extension of both knees and the toes of the right foot There were no trophic changes Patellar reflexes were normal the Achilles reflexes were weak There was hypesthesia algesia of both lower legs MCV of the right peroneal nerve was 43 m/sec EMG showed slight loss of motor units in the right rectus

Table I *Blood sugar ophthalmoscopy and neurological findings in a group of diabetic patients with muscular atrophy and weakness*

Unilateral and bilateral paresis may occur in different groups of muscles in the same patient
The same is true of regression and progression

Case no	Age (y)	Dur of d ab (y)	Retpathy	24-h blood sugar level (mg)	Sens defect	Pain	Paresis				Clin effect of treatment		MCV (m sec peron nerve)	Pathol EMG		VPT great toe (volts)
							Prox	Dist	Bilat	Unilat	Regress	Progress		Paretic muscle	Non par muscle	
1	27	7	–	150	+	+	+	–	+		–		33	+		30
2	30	14	+	175	+		+		–				38		+	13
3	44	35	+	400	+			+		+	–		42	–	–	44
4	38	7		un stable	–			+	–				33	–		>50
5	5	½	–	180	+	+		–		–			0	–		[illegible]
6	56	0		300	+	+	+	+	–				31	–		>50
7	6	½	–	150	+	+	+	–	+	+			0	–	–	[illegible]
8	56	0		150	+	+	–			–	–	+	43	–	–	50
9	67	4	–	200	+	+	+	+	+			+	30	–	–	50
10	51	½	–	00	–		+	+	+		+	+	0	+		[illegible]
11	57	0	–	250	–			+		–	+		45	–	+	40
12	61	6		100	+	+	+	+	–	–		–	40	–	–	24
13	70	10	–	170	+	+	+	+		–		–	40	–		50
14	63	8	+	100	+	+	–		–		+		42	–	+	19
15	50	24	+	un stable	–		+	–	–			–	41		–	23
16	63	15	+	230	–		+	–	+	–			43	–		50
17	64	1	+	200	+			+	+				3	–		50

femoris muscle severe loss in the right extensor dig. brevis VPT >50 volts on the great toes

Eighteen months later the patient's metabolic status was unchanged Subjectively there was increased weakness in the legs, but the objective findings were unchanged There was now atrophy of the right quadriceps and both the patellar and the Achilles reflexes were weak There was reduced sensibility in both lower le and the right thigh. MCV in the right peroneal nerve was 36 m se EMG and VPT were unchanged

Case 17

A 64 year-old woman who had been diabetic for 21 years. She had been treated with insulin On admission the blood sugar level was around 200 mg 100 ml. Severe retinopathy Neurological examination revealed *weakness* in the extensors of the toes *atrophy* of both extensor dig. brevis muscles, deep areflexia, hypesthesia algesia of both lower legs MCV in the peroneal nerve wa 3_ m sec EMG of the extensor dig. brevis muscle showed marked loss of motor units VPT >50 volts on the great toes

DISCUSSION

The most common clinical findings in diabetic neuropathy are as is well known asymptomatic deep areflexia and a distal defect of sensation in the lower extremities Pareses are relatively rare although neurophysiological studies have revealed that the motor system is affected from the very start of diabetes (8 9)

According to Garland's most recent definition (7) diabetic amyotrophy is a purely motor syndrome almost invariably asymmetrical proximal muscles tend to be most involved appropriate tendon jerks are depressed or absent pain is usually felt in the region of the affected muscles" He maintains that the syndrome is the result of poor diabetes regulation and that it is completely reversible with adequate treatment of the diabetes Diabetic retinopathy was not seen in connection with the syndrome in the 30 cases reviewed by Garland.

The 17 cases observed in the present series presented a clinical picture of neuropathy and all had paresis and muscle atrophy The neuropathy must be regarded as of diabetic origin in all cases except one (see later) In no case was there any suspicion of avitaminosis or a large alcohol consumption None of the patients had an elevated

with the exception of dysesthesia of the first toe inter stitium on the right foot. MCV in the right peroneal nerve was 33 m/sec as against 47 m/sec on the left side EMG in both extensor dig brevis muscles and the right tibialis anterior muscle showed severe loss of motor units EMG from the right rectus femoris muscle revealed no abnormalities VPT was >50 volts on both great toes

Treatment with tolbutamide was reinstituted and the paresis again disappeared in the course of three months MCV EMG and VPT changed only slightly during the following year

Case 12

A 61 year-old woman with diabetes of six years duration Diet was the only treatment given during the first five years One year before admission instability developed in the right leg Because of poor diabetes status and a weight loss of 25 kg insulin therapy was started Shortly thereafter weakness of the left leg and pain in both thighs developed On admission the blood sugar level was normal there was diabetic retinopathy Neurologic examination revealed marked *atrophy* and *weakness* of both quadriceps muscles deep areflexia and hypesthesia algesia in the lower legs EMG from the quadriceps muscle showed a severe loss of motor units and from the tibial anterior muscle a mild loss of motor units

The patient was seen in the diabetes clinic one year later she had discontinued insulin and again had appreciable glucosuria The neurological symptoms and signs were unchanged however there was now paresis of the left foot s dorsiflexors in addition MCV in the left peroneal nerve was 40 m/sec EMG from the left extensor dig brevis muscle showed a severe loss of motor units VPT on the great toes was 24 volts

Case 13

A 70 year-old woman with diabetes for ten years She had been treated with insulin during the first four years There had been a period of lancinating pains in the left leg and weakness on extension of the left knee at the time of the appearance of diabetes Half a year before examination she had lancinating pains in the entire right *leg and weakness on extension of the right knee Insulin* treatment was again started At the time of examination the blood sugar level was about 170 mg/100 ml There was diabetic retinopathy Neurologic examination revealed slight *atrophy* of both quadriceps mild *weakness* on extension of the right knee and dorsiflexion of the right foot and toes There was deep areflexia in the legs reduced sensation for pin prick and touch in both feet MCV in the right peroneal nerve was 40 m/sec EMG from the right rectus femoris muscle showed mild loss of motor units from the right tibialis anterior muscle interference denervation potentials from the right extensor dig brevis muscle severe loss of motor units VPT was >50 volts on both great toes

The patient was seen eight months later The blood sugar level was normal There was weakness of both quadriceps muscles and both tibialis anterior muscles and hypesthesia of both lower legs MCV in the right peroneal nerve was 30 m/sec EMG from the right rectus femoris muscle showed mild loss of motor units from the right tibialis anterior muscle and extensor dig brevis muscle severe loss of motor units VPT was unchanged

Case 14

A 63 year old woman who had had diabetes for eight years The patient neglected control and treatment during the first 7 ¹/₂ years and only during the last ¹/₂ year before admission was she treated with chlorpropamide During the year before admission the patient had lancinating pains in the thighs and trouble in walking On admission the blood sugar level was normal There was *retinopathy Neurologic examination revealed* marked *atrophy* and *paresis* of both quadriceps muscles deep areflexia in the legs hypesthesia algesia from the axillae downwards MCV in the peroneal nerve was 4? m/sec EMG of the quadriceps and extensor dig. brevis muscles showed slight loss of motor units VPT on the right great toe was 19 on the left great toe >50 volts

The patient was seen on several occasions during the following two years during which time a gradual subjective and objective improvement in strength in the lower extremities was seen The pains disappeared and sensation was reported as normal Deep areflexia in the le s persisted MCV EMG and VPT remained essentially unchanged

Case 15

A 50 year-old man with diabetes for 24 years who had been treated with insulin with frequent episodes of insulin shock One year before admission progressive weakness in both arms began On admission the blood sugar level was found to be quite variable There was retinopathy Neurologic examination revealed diffuse *atrophy* and *weakness* of the musculature of the shoulder girdle and upper extremities There were fasciculations in both arms and legs The biceps reflexes could be elicited but there was otherwise deep areflexia Sensation was normal MCV in the ulnar nerve 57 m/sec in the peroneal nerve 41 m/sec EMG of the deltoid muscles and adductor pollucis showed a severe loss of motor units and fasciculation potentials EMG of the quadriceps and extensor dig brevis muscles revealed nothing abnormal VPT on the index finger was 10 volts on the great toes 23 volts

On readmission ¹/₂ year later progression of the paresis in the upper extremities was noted There wa no involvement of the cranial nerves EMG was unchanged. The patient died at home of pneumonia two years after the first admission

Case 16

A 63 year old woman with a duration of diabetes of 15 years Treated with insulin. During the last three years a subjective weakness in the upper extremities was noted On admission the blood sugar level was about 230 mg/100 ml There was retinopathy Neurologic examination revealed mild *weakness* on extension of both knees and the toes of the right foot There were no trophic changes Patellar reflexes were normal the Achilles reflexes were weak There was hypesthesia algesia of both lower legs MCV of the right peroneal nerve was 43 m/sec EMG showed slight loss of motor units in the right rectus

of diabetic retinopathy and loss of sensibility in diabetic amyotrophy The question therefore arises whether so called diabetic amyotrophy is associated with an adequate number of characteristics to permit the designation of a special syndrome distinct from the more usual feature of diabetic neuropathy the symmetrical distal sensory one

First it could be asked whether there is any evidence that distal neuropathy in diabetes mellitus is essentially different from proximal?

It has earlier been demonstrated that the distal symmetrical sensory diabetic neuropathy is very often accompanied by a subclinical motor affection (8 9) Sudden distal pareses are occasionally seen with the same tendency to recovery as proximal ones On the other hand pain is seldom seen in connection with isolated distal pareses

Proximal clinical findings in diabetic neuropathy tend to be purely motor although the occurrence of pain must mean that some sensory disturbance is taking place As the mechanism of sensory perception is not well known it cannot be denied that proximal pain might be a reflection of the same underlying process which gives dysesthesia or loss of sensibility more distally In our experience proximal findings are or become as symmetrical as distal Even in the absence of clinical findings it is conceivable that lesions are in progress in the proximal muscles as is known to occur more distally In a consecutive series of autopsy investigations of 15 juvenile long term diabetics only four of whom had clinically demonstrable weakness a proximal muscle the semimembranosus was studied and it was found that neurogenic atrophy was present in every case (15) Save Soderberg and Angervall (18) studied nine long term diabetics between the ages of 29 and 63 years at autopsy and found demyelinisation of the femoral nerve and atrophy of muscle fibers in the quadriceps muscle in all cases None of these patients had presented muscle atrophy or clinically demonstrable paresis before death. It could thus be concluded that proximal pareses are not in reality acute catastrophies but probably the result of a long-standing subclinical affection as can be seen more distally

Next to the problem of eventually existing differences between proximal and distal neuropathy is the question of other evidences of a pure or predominantly motor neuropathy than mere clinical observations

Several studies suggest that there is no elective abnormality of the motor ganglion cells or ventral roots in diabetic paresis (7 12 16) One of the differential diagnostic difficulties one runs into if one accepts Garland's definition of a pure motor syndrome is amyotrophic lateral sclerosis The only one of our patients in whom the EMG indicated an affection of the anterior horn cells (no 15) probably suffered from the disease In all other cases the EMG both proximally and distally suggested peripheral neurogenic paresis We have not come across a myogenic picture as seen by Wiesendanger and Bischoff (19) in a few cases

It is true that motor affections in diabetics often run a more acute or transitory course than the more insidious and slowly progressive sensory disturbances However clinical improvement or cure of motor affections is in general only apparent Repeated measurements of MCV and EMG in the patients here presented showed only slight changes in spite of subjective or objective improvement in motor abilities Garland has also mentioned that "muscle atrophy and electromyographic changes may be the last feature to return to normal" (7)

There may be two ways to explain why pareses appear or suddenly become worse Firstly as Sullivan suggests (17) a vascular catastrophy may occur in a nerve vessel or secondly it might be that a prolonged abnormal metabolic influence at some point exceeds a critical threshold Previous experiments in animals have shown that motor nerves can be affected by the metabolic status (3 14) and that this can occur in humans has also been confirmed (10) Diabetics with pareses are usually older patients with a long or unknown duration of diabetes Many present evidence of diabetic angiopathy or are metabolically poorly controlled

In conclusion it may be said that there is no explanation so far of the occasional discrepancy in the progress of sensory and motor affections in diabetes mellitus However sensory and motor clinical neurophysiological and pathological findings occur intertwined in the same type of patients Topographic differences are unlikely to exist It therefore seems most natural to regard muscular weakness and wasting in diabetics as an expression of a widespread peripheral neuropathy—and not a special syndrome even if sensory disturbances may not be demonstrated at the same time

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STUDIES ON PLASMA ANGIOTENSINASE ACTIVITY IN NORMAL AND TOXEMIC PREGNANCY

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Abstract The behavior of plasma angiotensinase activity has been studied in normotensive pregnant women and in toxemic patients during pregnancy and in the puerperium

In normal pregnant women a slight increase of plasma angiotensinase activity occurred in the third trimester with a greater increase at the time of delivery It rapidly normalized during the puerperium.

In toxemic patients in the third trimester of pregnancy and at the time of delivery the increase of plasma angiotensinase activity was more marked than that observed in normal pregnancy The activity was also significantly higher for several days during the puerperium

Examination of our results reveals a possible correlation between plasma angiotensinase activity and endogenous angiotensin production The significance of renin angiotensin system involvement in normal and toxemic pregnancy is discussed

Investigations by several authors suggest involvement of the renin angiotensin system in normal and toxemic pregnancy

An increase of renin and angiotensin plasma levels occurs in normal pregnant women (6 11 35) In the toxemic patients Brown et al (8) have found an increase of plasma renin In no case however did this level exceed that observed in normal pregnancy Meriel et al (27) have noted in the same patients an increase of plasma angiotensin level Also it is of interest to note that Itskovitz et al (16) have found in a toxemic patient an increased granularity of the juxtaglomerular cells which seem to be the site of renin production On the other hand Pickering (33) and more recently Assali (2) stressed the importance of renin angiotensin system involvement in the etiology of specific hypertensive disease of pregnancy Lastly an eclampsia like syndrome has been produced in rats by administration of renin in conjunction with steroids and sodium chloride (26)

Circulating angiotensin is destroyed by one or more enzymes present in the blood A peptidase with high degree of specificity for angiotensin occurs in normal human plasma and red cells This enzyme angiotensinase A requires α L aspartic acid or α L asparagine as the N terminal amino acid in its angiotensin substrate (18) Klaus et al (20) considered an aspartic acidaminopeptidase system responsible for angiotensin degradation but conceded the participation of other aminopeptidases However a specific enzyme has not yet been identified it is necessary therefore to refer to plasma angiotensinase activity"

Plasma angiotensinase activity has been studied in hypertension (14 21 24 30 39 40) and in conditions commonly associated with secondary hyperaldosteronism such as cirrhosis with ascites nephrotic syndrome and congestive heart failure (5 14 21 22 28 29)

Of particular interest is the study of this activity in normal and toxemic pregnancy in relation to the presence of respectively marked secondary hyperaldosteronism and arterial hypertension However investigations made in this field have produced different results In normotensive pregnant women Klaus (19) and Vorherr et al (38) have not found any significant variation of plasma angiotensinase activity while Page (31) and Hickler et al (14) report a marked increase in the third trimester of pregnancy Landesman et al (23) have noted a slight increase during the ninth month and on the day of delivery In toxemic patients Page (31) Hickler et al (14) and Landesman et al (23) report an increase of

this activity while Vorherr et al (38) have observed no significant variation

In consideration of the different results mentioned above and the interest of several authors in this subject we propose to study the behavior of the plasma angiotensinase activity in an adequate series of women in normal and toxemic pregnancy during the labor and the puerperium

MATERIAL AND METHODS

Observations were made on 43 normotensive pregnant women 8 in the first 8 in the second and 27 in the third trimester Twenty three of the latter were examined at the time of delivery Further observations were made also in the puerperium 17 on the first day 22 on the fifth day and 10 on the tenth day For comparison 20 toxemic patients were examined in the third trimester of pregnancy Eighteen of these were controlled before delivery 13 during the delivery 17 on the first day of the puerperium 16 on the fifth day and 11 on the tenth day All toxemic patients presented different degrees of arterial hypertension proteinuria and edema Eclampsia was present in two of these patients Finally 20 normal non pregnant women—between the ages of 20 and 40—were examined as controls

Plasma angiotensinase activity was determined by a biologic method of Landesman et al (23) and Biron et al (5) partially modified This method is based on the degradation of pressor activity of synthetic valine 5 angiotensinamide by untreated plasma

1 Ten milliliters of venous blood of normal non pregnant women of normal pregnant women and of toxemic patients were placed in heparanized tubes and centrifuged Blood samples showing evidence of hemolysis were discarded Plasma was then frozen in two or more tubes and kept at −5 C for as long as one month

2 The angiotensin solution was prepared containing 2 μg of valine 5 angiotensinamide to 1 ml of 0 1 *M* sodium phosphate (pH 7 4) which also had been stored at −5 C

3 The plasma samples and angiotensin solution were thawed not more than 1 h prior to incubation and brought to 37 C in a water bath Plasma 0 75 ml from each patient s sample was added to 0 75 ml of the angiotensin solution and incubated at 37 C for 10 min The reaction was stopped by boiling for 2 min

4 An absolute control was obtained by boiling the patient s plasma immediately after mixing it with angiotensin solution

5 Residual angiotensin was estimated immediately on a blood pressure preparation Male albino rats of Wistar type weighing 300–350 g were used The rats were anesthetized with nembutal (40 mg/kg) intraperitonally esametonium was injected subcutaneously (5 mg/kg) then the rat was tracheotomized The jugular vein was cannulated with polyethylene tubing connected to a microsyringe for injecting samples and the carotid artery was linked to a mercury manometer with normal saline filled tubing

6 The sequence of injections was as follows 3 injections of the control sample 2 injections of the normal sample 1 injection of the control sample 2 injections of the patient s sample (pregnant normal women or toxemic patient) 1 injection of the control sample The degree of inactivation by the normal sample was considered to represent 100% angiotensinase activity and whatever increased or decreased degradation was found in the patient s sample was expressed as a percentage value over or under that for 100 Calculations were made using the following formula

$$\frac{\text{mm Hg control mm Hg patients}}{\text{mm Hg control}} \times \frac{\text{mm Hg control}}{\text{mm Hg control mm Hg normal}} \times 100$$

All assays were done in duplicate the second time a different rat was preferred Angiotensinase activity of the same subject during pregnancy at the time of delivery and during the puerperium was determined with the same blood pressure preparation In this way it was possible to obtain an easier comparison of the values determined under the same experimental conditions

According to Biron et al (5) and Landesman et al (23) the normal range as determined from a series of 20 normal non pregnant women assayed against each other was 80 to 120 % Reproducibility of the assay on any given plasma varied within ±15

RESULTS

The results observed in normotensive pregnant women are shown in Table I Plasma angiotensinase activity in the first and second trimester of normal pregnancy is within normal limits on the other hand a slight tendency to increase has been observed in the third trimester At the time of delivery this activity is constantly higher than that noted at the end of pregnancy The plasma level increases by an average of 97 7 in the first to 107 7 in the second to 121 3 in the third trimester and to 149 5 at the time of delivery On the other hand a marked decrease already evident on the first day is observed during the puerperium

A significant increase of plasma angiotensinase activity has been found in the third trimester of toxemic pregnancy (Table II) and a further increase occurs at the time of delivery This activity is still marked in most cases during the puerperium The increased level averages 161 7 in the third trimester 178 9 at the time of delivery 154 4 on the first day of the puerperium 134 6 on the fifth day and 141 3 on the tenth day The observations on the tenth day are limited because some patients were discharged after the

Table I *Plasma angiotensinase activity in normal women during pregnancy labor and the puerperium*

Cases examined	Age	Plasma angiotensinase activity						
		Pregnancy				Puerperium		
		1st trim	2nd trim	3rd trim	Labor	1st day	5th day	10th day
1 S L.	40	87 6						
2 A M	42	91 0						
3 S M	36	90 0						
4 G M	25	97 9						
5 M T	33	103 2						
6 M G	35	1?4 9						
7 S R	32	90 2						
8 M M	?8	97 1						
9 A G	?0		107 8					
10 D D	42		105 2					
11 S M	40		91 5					
12 O R	28		111 3					
13 P C	39		133 2					
14 D M	32		113 4					
15 S A	25		103 1					
16 D S	?9		96 5					
17 N E.	31			133 0				
18 M L	30			134 6				
19 U G	33			133 5				
0 S A	28			125 1				
?1 P G	31			130 1	135 5	108 9	80 5	115 2
?2 B D	30			138 0	164 4	88 3	110 0	115 0
23 R V	27			111 7	116 2	110 6	111 7	95 7
?4 B M	25			101 6	137 5	90 7	85 3	90 1
25 M R.	26			123 7	128 2	108 0	108 0	
26 D C	28			137 1	133 2	103 8	93 7	96 4
27 Z. L	39			126 4	146 2	102 0	90 6	
8 S G	28			119 7	151 8	95 3	102 5	
29 V R	24			89 6	135 6	107 5	9? 1	
30 M M	21			116 4	139 6	119 3	81 4	
31 M G	24			143 3	159 7	120 7	120 7	101 6
3 D C	24			118 4	138 1	87 4	101 4	
33 P M	20			119 5	142 5	86 0	89 0	89 0
34 S A	27			125 1	196 5	121 1	108 3	
35 S L.	34			125 6	160 4	81 2	74 8	
36 F R	24			132 9	18? 8		98 7	
37 M S	30			121 7	158 4	110 9	110 9	103 1
38 D R	34			120 4	161 7	109 0		
39 C T	25			10 8	136 4		93 .	
40 N G	36			116 5	143 9		119 5	
41 C G	31			108 5	151 1		0 8	78 1
4? C D	24			112 8	147 6		76 5	93 1
43 C G	26			108 4	17 1		91 4	
Mean		97 7	107 7	121 3	149 5	102.9	96 0	97 7
Change in relation to normal values ( )		- . 3	- 7 7	- 21 3	- 49 5	- 9	4 0	- 2 3

disease—at least from the clinical point of view—had totally regressed.

An intrauterine fetal death occurred in case 3 following which the toxemic symptoms quickly regressed It is interesting to note that in this patient plasma angiotensinase activity normalized when the fetal death occurred before delivery

DISCUSSION

The data reported indicate that plasma angiotensinase activity slightly increases in the third trimester of normal pregnancy with a greater increase at the time of delivery and rapidly returns to normal during the puerperium

In the third trimester of toxemic pregnancy

Table II *Plasma angiotensinase activity in toxemic patients in the third trimester of pregnancy during labor and the puerperium*

Cases examined	Age	Plasma angiotensinase activity				
		Pregnancy 3rd trim	Labor	Puerperium		
				1st day	5th day	10th day
1 P G	43	189 2	195 7	106 4	119 3	136 0
2 F A	29			183 6	171 2	191 5
3 C S	22	132 0	111 3		96 2	88 7
4 L M	27	140 5	195 9	149 6	153 3	158 2
5 T G	39	121 4	179 6	159 3	157 2	
6 C M	38	175 7	191 3	175 7	185 2	
7 S C	31	135 7		132 1	95 4	
8 S F	43	176 1		135 7	137 1	
9 C M	26	154 5	176 3		151 4	136 9
10 A M	37	152 1	176 3	151 1	141 4	
11 P G	32	131 1		176 8	103 6	
12 S G	31	214 8	207 9	120 1	94 7	107 7
13 C A	26	137 0	134 5	121 0		
14 M I	26	169 3	176 1	178 0	159 6	
15 M M	30	141 2	174 5	149 8	149 8	147 1
16 A M	37	155 4	157 5	149 1	102 1	90 0
17 M G	43	145 7		140 8	141 2	140 5
18 D M	43	180 [illegible]	249 7	279 4		211 2
19 P G	40	259 1				147 1
20 P D	26			274 4		
Mean		161 7	178 9	154 8	134 6	141 3
Change in relation to normal values ( )		+ 61 7	+ 78 9	+ 54 8	+ 34 6	+ 41 3

this activity markedly increases with an even greater increase at the time of delivery The reduction of enzymatic activity during the first ten days of the puerperium is notably less in these patients than in the normal pregnant women (Fig 1)

These results are in agreement with those of Landesman et al (23) and Hickler et al (14) while Page (31)—using an impure preparation of angiotensin—did not observe a greater increase in toxemic pregnancy than in normal pregnancy

Hickler et al (14) presented an interesting working hypothesis According to them the states which may have in common an underlying increased rate of angiotensin elaboration with or without hypertension may develop an increased plasma capacity to inactivate angiotensin through a process of substrate induction that is through a specific enzymatic adaptation at the cellular level In agreement with this hypothesis Szabo and Magyar (37) have observed an increased plasma angiotensinase activity in dogs both during experimental shock—consequent on the activation of the renin angiotensin system—and after the injection of high doses of synthetic angiotensin The authors suggest that probably as a result of some feedback mechanism high levels of circulating angiotensin increase angiotensinase production or release

Therefore if such enzymatic activity reflects production of angiotensin an increased activity of the renin angiotensin system may occur in the third trimester of normal pregnancy at the time of delivery and particularly in the toxemic pregnancy Reports by these authors concerning the blood levels of renin and angiotensin (6 8 11 27 35) and the increased granularity of the juxtaglomerular cells in a toxemic patient (16) concur with this hypothesis

Recent investigations indicate the presence of a renin like substance in the uterus and placenta of rabbits (13 42) in human placenta (15) and in human amniotic fluid (7) Hodari et al (15) suggest that pressor activity found in the placenta is due to angiotensin and that a renin like enzyme must be present in the placenta to liberate this angiotensin However the detection of a renin like substance in the placental fetal uterine

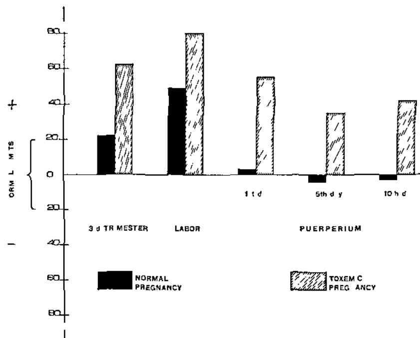


Fig 1 Average per cent changes of plasma angiotensinase activity in normal and toxemic pregnancies

complex does not permit the conclusion that the placenta produces this enzyme its presence may represent only storage in the placenta due to the capacity of this organ to concentrate it (15 36)

Concerning the significance of possible increased activity of the renin angiotensin system in normal pregnancy it seems reasonable to assume that *this phenomenon is responsible for the* secondary hyperaldosteronism of pregnancy because this system—according to present knowledge—is the most important humoral regulator of aldosterone secretion Greater production of progesterone in pregnancy causes an increased excretion of sodium sodium depletion as is well known is capable of increasing plasma renin levels and *hence of stimulating aldosterone secretion* (6 25) Progressive pressure on the inferior vena cava due to the pregnant uterus likewise may be responsible for increased plasma renin levels Kerr et al (17) demonstrated that the vena cava was completely obstructed in 10 of 12 supine women in late pregnancy Moreover Yankopoulos et al (41) and Davis et al (10) demonstrated that ligature of the *inferior vena cava* causes secondary hyperaldosteronism in experimental animals This hypothesis is indirectly confirmed by the increased plasma angiotensinase activity during labor since at this time uterine pressure on the inferior vena cava is notably augmented Our observations of plasma angiotensinase activity were made during the last stage of labor or at the time of delivery

Concerning the notable increase of plasma angiotensinase activity in toxemic patients some other factors have to be examined In toxemia *there are peculiar pathological lesions of renal* afferent arterioles and glomeruli which persist during the puerperium for varying intervals (1 12 34) Consequently there is a reduction of blood flow and glomerular filtration (3 4) Because of this reduction of blood flow in the afferent arterioles juxtaglomerular cells may be stimulated to produce renin Renin liberates angiotensin *and its presence is indirectly demonstrated* by increased plasma angiotensinase activity

While probably not the primary factor in the etiology of toxemia, involvement of the renin angiotensin system may be a secondary mechanism in the development and maintenance of the disease This capacity seems to be a consequence of the hypertensive action of angiotensin and of *its ability to stimulate production of aldosterone* with sodium retention and—according to recent investigations (9 32)—of catecholamines

During the puerperium of normal pregnancy plasma angiotensinase activity rapidly decreases

while during the puerperium of toxemic pregnancy this activity is still elevated but lower than that found in the third trimester It seems reasonable to assume that this phenomenon is related to the persistence during the puerperium of the renal vascular alterations previously mentioned and therefore to prolonged stimulation of the juxta glomerular apparatus The persistence of these peculiar anatomical and functional lesions ap parently causes a prolonged production of renin by the juxtaglomerular cells

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THE PROGNOSIS OF CONSERVATIVELY TREATED DIABETIC GANGRENE

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Abstract One hundred and fifty-seven patients have been admitted to the medical ward in the years 1961 to 1967 inc usive in whom a cons rvative treatment for diab tic gangrene of the lower extremities has been attempted They have been followed for an average observation time of 3 8 years Clinical information has been obtained con cerning 100 of the patients The survival of the pa tents in the material is shown to be considerably less than for the population at large with only approximately 50 % of the patients surviving for more than three years The excess deaths were caused mainly by arterio sclerotic heart disease and to a lesser degree by com plications of the gangrene The attempted conservative therapy has been very time consum ng and amputations could not be avoided in the majority of cases It is shown however that the mutilating calf and thigh am putation can be avoided in approximately 60 of the patients About one third of the patients who have had an amputation on one side will require an amputation on the second initially unaffected leg within three years after the first amputation The social outlook for the patients is shown to be poor Only a few of them con tinued to be ga nfully employed and 25 were at follow up bound to a wheel chair or under permanent care in an institution.

Gangrene of the lower extremities is a serious complication of diabetes mellitus Often amputa tion cannot be avoided and the more extensive the amputation the more likely it is to lead to disability of the patient As a consequence of this fact a conservative treatment of gangrene in pa tients with diabetes has been attempted at Bispe bjerg Hospital since 1961 The purpose of the present study is to evaluate the results of the treatment with respect to the survival of the pa ti nts the fate of the extremities and the social fate of the patient The study comprises 157 pa tients admitted to Medical Department C Bispe b erg Hospital in the period 1961 to 1967 with diabetic gangrene of the lower extremities All patients have been followed up in January and February 1968

MATERIAL

The study compris s all patients admitted between Janu ary 1 1961 and December 31 1967 to Medical Depart ment C Bispebjerg Hospital because of gangrene of the lower extremities as a complication of diabetes The d stribution according to age sex and duration of dia betes is seen in Table I In eight patients diabetes had been diagnosed before the age of ?0 years Of the 157 patients 46 had previously been admitted to hospital be caus of gangrene and 31 of them had undergone am putation

Treatment during initial hospitalization in the pe iod 1961 to 1967

During their period in hospital 14 patients were treated by diet alone 44 with tolbutamide or phenformin and 99 with insul n Wound infections were treated for one to six weeks with antibiotics accord ng to the sensitivity of the infecting agent The local treatment of the gan grene was managed by the orthopaedic surgeons O J Gottlieb and J Nielsen M D and amputations were per formed only when it was obvious that the affected part could not be preserved by conservative treatment In 66 cases no amputations were performed in 91 there was a total of 111 amputations 18 of which were reoperations because of too conservative initial amputation Twenty-one patients left the hospital as double amputees 19 had been amputated unilaterally on a previous occasion while two were amputated bilaterally during this admission

During and after hospitalization a phys otherap st a prosthetist and a social worker participated in the reha bilitation of the patient who often had to be followed as outpatient for as long as a year before their ulcera tion had healed Of the 133 discharged patients 10 were bound to a wheel chair The average length of hospitaliza tion was 77 days During the initial period in hospital 24 patients d ed These patients will be dealt with later

Follow up study

The patients were followed up in the period between January 1 and February 2 1968 The average observation time was 3 8 years All 157 pat ents were traced A total of 74 patients had died (24 of whom during the initial hospitalization) and their fate has been determined through information from hospital records death certif cates and information from their fam l es Autopsy re ports were obtainable for 51 patients The rema n ng 83 patients were examined by the author

Table I *Age and known duration of diabetes mellitus at first admission for gangrene of the lower extremities between 1961 and 1967 inclusive*

Age	Duration of diabetes in years 0–1		1–5		6–14		≥15		Total	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
≤49	3	0	0	0	4	0	5	3	12	3
50–59	3	2	4	5	5	3	5	4	17	14
60–69	7	7	8	3	7	6	12	5	34	21
70–79	6	4	3	1	9	7	11	3	29	15
≥80	0	1	1	2	0	3	2	3	3	9
Total	19	14	16	11	25	19	35	18	95	62

METHODS

On the basis of statistical information on the death rate in the Greater Copenhagen Area (10) the expected survival rate has been calculated for the patients in this study (one year age groups have been used) The expected causes of death in the study have been estimated on the basis of statistical information on the causes of death in the Greater Copenhagen Area in the year 1963 (1) in the age groups 45 to 65 years 65 to 85 years and over 85 years The actual survival rate the employment rate and the amputation rate for the patients in the material have been found by the life table method (7) for calculation of the probability of death unemployment and amputation in each observation year

RESULTS

Mortality

A total of 74 patients (43 men and 31 women) of the 157 patients had died Table II shows the expected and observed numbers of deaths for men and women in each group The excess mortality was highest in the younger age groups and higher for women than men Fig 1 shows the expected and observed survival rates Only 50% of the women and 55% of the men survived longer than 3 years In Table III the expected and observed causes of death in the material are compared The number of deaths from vascular disease is markedly higher than expected arteriosclerotic heart disease being by far the most frequent cause while the number of deaths from neoplastic disease is much smaller than expected In no case could death be attributed to a surgical procedure In five cases the cause of death was septicaemia due to the same infecting agent as found in the gangrene Five patients died from pulmonary embolism all of whom were bedridden because of gangrene or amputation Thus 10 of the 74 deaths could directly be related to the gangrene

Table II *Expected and observed deaths in observation period for 95 men and 62 women by age groups*

Age (y)	No of pats	Expected deaths in observation period	Observed deaths in observation period
Men			
≤49	12	0 3	3
50–59	17	0 95	5
60–69	34	5 65	18
70–79	29	7 52	14
≥80	3	1 09	3
	94	15 53	43
Women			
≤49	3	0 03	0
50–59	14	0 49	6
60–69	1	1 83	8
70–79	15	2 79	11
≥80	9	2 35	6
	62	7 49	31

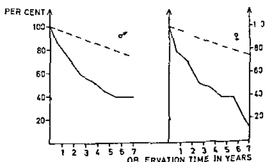


Fig 1 Expected and observed survival rate and observed employment rate for 95 men and 62 women with diabetic gangrene of the lower extremities (Expected survival rate – – observed survival rate —— employment rate)

Table III *Comparison of the expected and observed causes of death*

	Men		Women	
	Expected no of deaths	Observed no of deaths	Expected no of deaths	Observed no of deaths
Arteriosclerotic heart disease	12 1	23	7 4	17
Cerebrovascular accidents	3 7	3	3 9	3
Neoplastic diseases	12	2	9 2	1
Uraemia chronic kidney infections	0 4	5	0 8	1
Pulmonary infections—tb	0 9	6	1 2	[a]
Pulmonary embolism	0 7	1	0 5	4
Septicaemia	0 002	2	0 05	3
Suicide	0 6	1	0 4	0

[a] Statistical information uncertain

Length of hospital stay

The average length of the initial stay in hospital was 77 days During the following observation period the average rehospitalization time for 157 patients because of gangrene was 69 days Thus the total average hospital stay for each patient was 146 days For the 157 patients the mean observation time from the initial admission until death or follow up was 922 days of which 146 (15°) were spent at hospital

Amputations

On the basis of the amputations made during the initial period in hospital and later course of the disease an amputation rate has been calculated for all types of amputations and for calf and thigh amputations separately (Fig 2) The amputation rate is approximately the same for men and women In the age groups young r than 60 years the calf and thigh amputation rates were a little lower than in the age groups older than 60 years Many patients were admitted with gangrene of one leg and an apparently healthy second leg but during the course of the diseas th y became double amputees Only those patients who had been amputated on the primarily affected leg had amputations performed on the second leg The amputation rate on the initially unaffected leg is seen in Fig 3

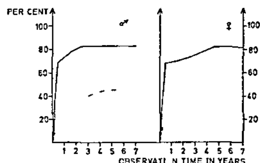


Fig 2 Amputation rate for all types of amputations (——) and for calf and thigh amputations ( ) in 95 men and 67 women

Abilities of the patients at follow up

Of the 83 patients followed up 22 were bound to a wheel chair or under institutional care Non of the patients with bilateral femoral or crural amputations and only two-thirds of those with unilateral femoral or crural amputations could walk at the follow up with the help of a pro the sis

Employment

At the initial admission only 40 ° of the men and 23° of the women were gainfully employed These low percentages are partially due to the high number of old patients in the material and also to the disability from complications of the diab tes On the basis of the observations an employment rate has been calculated (Fig 1) It is se n that very few women were re-employed after the initial admission and for the men too the perc ntage of employed decreased rapidly

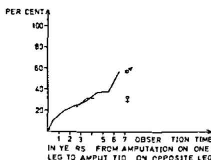


Fig 3 Amputation rate on second primarily unaffected extremity

DISCUSSION

The life expectancy of patients with diabetic gangrene is short Silbert (9) found that only 40 % of patients who had undergone amputation for diabetic gangrene lived for more than three years The short life expectancy was also seen in this study approximately only 50% of the patients surviving for more than three years The mortality in this study was considerably higher than for the diabetic population at large (4 6 8) but as in the general diabetic population (4) the excess mortality was highest in the young age groups and higher among women than men

The high mortality was as expected mainly due to vascular disease (3 4 6) with arteriosclerotic heart disease accounting for over 50 % of the deaths

In a previous report (2) on patients who received mainly surgical treatment for diabetic gangrene the average hospital stay was found to be 26 days In the present study the average initial time in hospital was 77 days and the total average hospital stay 146 days which shows how costly and time consuming conservative treatment of gangrene is

The main reason for attempting a conservative treatment of gangrene is the difficulty for the patient with a calf or thigh amputation to learn to use a prosthesis In this study it has obviously not been possible to avoid amputations since the amputation rate for all types of amputations after 3 years of observation was approximately 80 % However only approximately 40 % required calf or thigh amputation and it thus seems possible to avoid these mutilating amputations in the majority of patients The frequent need of amputation of the second initially unaffected leg in patients unilaterally amputated has been pointed out by Goldner (5) In this study after three years of observation the amputation rate on the second leg was 30 % The poor fate of the second leg stresses the importance of avoiding mutilating calf and thigh amputations in the first leg as it is virtually impossible for the patients in the age groups dealt with in this study to walk after bilateral amputation of this type

The working capacity of patients with diabetes is judged to be only little inferior to that of the population at large (6) This is not so for patients with diabetic gangrene Only a minority of the patients are reemployed after the initial admission and their number decreases rapidly with the observation time Not only was the vast majority of the patients not able to work but 25 % of them depended on help from others being either wheel chair patients at home or under permanent care in an institution

On the basis of the findings in this study it seems safe to conclude that a considerable conservatism with regard to calf and thigh amputations is justifiable and also that little is gained by hesitating to perform minor relevant amputations in patients with diabetic gangrene

ACKNOWLEDGEMENT

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NUTRITION BY INTRAPERITONEAL FAT INFUSION

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Abstract A fat solution (Intralipid®) was administered intraperitoneally in 10 20% or 40% solution, to severely ill patients for nutritional purposes The fat infusions caused discomfort in 15 out of the 34 patients but it was of a mild nature in all except those who had undergone peritoneal dialysis on the day before the infusion Autopsy showed evidence of peritonitis in one patient death was due to cerebral haemorrhage In 1 cases no reactions of the peritoneum were seen at autopsy The rate of fat absorption estimated by a simple technique averaged at the least 3 (0-80) g per day which equals 790 small calories per day This is too low a calorie supply to allow the method to be recommended for routine use in severely ill patients

Glucose and saline are infused routinely into the peritoneal cavity in peritoneal dialysis Four thousand litres or more can be thus administered over a year to a uraemic patient without causing any serious damage to the peritoneum Fat has been infused into the peritoneal cavity on three indications (*a*) to try to prevent adhesions in the peritoneum in peritonitis (4) (*b*) to try to eliminate fat soluble poisons (1) and (*c*) to try to supply calories to severely ill patients (2) I have studied the reactions to and the absorption of intraperitoneally administered fat solutions

METHOD

Intralipid® (AB Vitrum, Stockholm) 00-500 ml in concentrations of 10 0 or 40% was infused through a coarse needle into the peritoneal cavity After local anaesthesia the needle was introduced through the skin about 3 cm caudal to the umbilicus in the midline The needle was connected to the infusion bottle via a drip set. As long as the fluid was only dripping in the drip set, the tip of the needle was situated subcutaneously As the needle was pushed through the fascia lata into the peritoneal cavity the fat solution began to flow through the drip set, showing that the tip of the needle was in the peritoneal cavity Five ml of Xylocain® were added to the solution, so as to cause less discomfort to the patient during the infusion Before infusion the infusion bottle was heated to 38 C in a water bath One thousand units of heparin were added to the fat solution to increase the absorption of fat from the peritoneal cavity

MATERIAL

Thirty four patients were given a total of 53 infusions. The main indication was to supply calories to severely ill patients who could not take any food themselves whose veins only with difficulty could be used for intravenous administration, and who were judged to be less suitable for nutrition through a gastric tube Some of the patients were motorially restless and pulled out the inserted gastric tube at every opportunity some offered violent resistance to the introduction of the tube and threw it up by vomiting, others had vomitings with the risk of aspiration Three uraemic patients were given fat intraperitoneally at the end of a peritoneal dialysis

RESULTS

The *reaction* to the fat infusion could not be assessed in 11 unconscious patients Nine patients experienced no discomfort four complained of slight discomfort eight of slight rapidly transient abdominal pain and three of great discomfort The latter were the three uraemic patients who were given the infusion at the end of a peritoneal dialysis reasonably the abdomen was tender after the peritoneal dialysis In none of the patients did palpation of the abdomen after infusion show signs of peritonitis There was no demonstrable difference between the reaction at 10 % at 20% and at 40 % solution One patient had a post infusion rise of temperature on three occasions the others showed no such reactions Each infusion lasted for 15 min In one case the infusion was given for 45 min to a sensitive patient he did not complain of any discomfort

Autopsy was performed in 14 cases on the 1st to 14th post infusion day In 12 cases no reaction was seen in the peritoneum Evidence of intracerebral haemorrhage lung emboli bronchopneu

Table I *Administered remaining and absorbed amounts of fat on 16 occasions after infusion of fat intraperitoneally*

Infused amount of fat (g)	Remaining amount of fat (approx g)	Absorbed amount of fat (approx g)	Duration (d)	Absorbed amount of fat per day (approx g)
100	20	80	1	80
100	2	98	2	49
100	63	37	2	19
150	140	10	2	5
80	44	36	3	12
200	130	70	3	23
200	90	110	3	37
200	75	125	3	42
200	40	160	3	53
300	300	0	5	0
200	18	182	5	36
80	17	63	5	13
200	2	198	5	40
200	1	199	6	33
200	1	199	7	28
800	370	430	12	35
200	2	198	13	15

monia and purulent peritonitis was found in one case an 82 year old man who had been in coma for more than a month He had reacted with fever to the peritoneal infusions but palpation of the abdomen had not elicited any signs of peritonitis His peritonitis had been suppressed by antimicrobic agents for a long time In one case the fat olution was slightly blood coloured

Absorption of fat from the peritoneal cavity was studied by a simple technique on 17 occasions On the 1st to 13th post infusion day th peritoneal cavity was filled with 2 or 3 l of rinsing fluid About 30 min after the end of infusion 1000–2000 ml of fluid were withdrawn through a stylet catheter inserted into th peritoneal cavity In one case only 20 ml were withdrawn through a thin catheter Samples for determination of fat in the peritoneal fluid were taken from the first and the last bottle in ten cases and from the bottles of fluid poured together in seven cases In two cases the fat content from the first bottle was much higher in three cases that from th last bottle was higher and in five cases the contents from the two bottles were almost equal

The amount of fat remaining in the peritoneal cavity was estimated by simple multiplication by the administered volume As the amount of administered fat was known the absorbed amount could be calculated approximately Through division by the number of days that the fat solution has been in the peritoneal cavity an estimate is obtained of the rate of absorption of fat from the peritoneal cavity in severely ill patients in whom intraperitoneal administration of fat would be indicated (Table I)

The sulphophosphovanilin reaction (5) was used *for the determination of the fat content of the* peritoneal fluid by the clinico chemical laboratory (head Lennart Jacobsson M D)

It will be seen from Table I that th absorption rate varied between 0 and 80 g per day No absorption was found in a uraemic woman with slight paralytic ileus and uraemic diarrhoea Absorption of 80 g in one day was found in a man with cerebral haemorrhage Five patients had absorped all the administered fat before the day of investigation these patients probably had a higher absorption rate than was noted here The absorption rate averaged at the least 32 g per day which equals 290 calories per day This is too low a caloric supply to allow the method to be recommended for routine use to severely ill patients The absorption is probably higher in less disabled patients but then on the other hand the indication for intraperitoneal infusion is probably less The absorption can possibly be increased by using solutions with smaller particles of fat or by infusing greater volumes

Fat is stored in the liver after repeated intravenous infusions of large amounts of fat solutions Liver damage has been reported in such cases The daily amount of fat solutions which can b given intravenously without risk of liver damage is not clear Cautious investigators recommend that not more than 100 g of fat should be administered intravenously daily (3) The suitable dose for intraperitoneal administration has not been estimated in this work because fat has only been administered for a few days

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ELECTROCARDIOGRAPHIC CHANGES IN CEREBROVASCULAR ACCIDENTS

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Abstract Electrocardiographic abnormal ties in various cerebrovascular accidents are described bas d on a clinical material The incidence of the ECG changes was 71 5% in the group with subarachnoid haemorrhage The figures for the groups with intracerebral haemorrha e and un classified cerebrovascular accidents were 57 1 and 41 1 respectively Attention has been focus d on pos tive ab normal T waves, and on S-T s gment e evations in con nection with an acute increase of blood pressure in the early phase after cerebrovascular accident. These changes were often shown to precede the T wave inversions which followed later Subendocardial haemorrhages and in on case distinct myocardial infarction were observed The authors believe that the prime factor responsible for ECG changes is an acute increas in left ven ricular pressure following elevation of intracranial pressure A short re view of the literature is also given

The first account of ECG changes (large up right T waves and prolonged QT intervals) in a patient with subarachnoid haemorrhage was pub- lished in 1947 (5) Not until 1953 however when Levine (17) reported on ECG changes attributed to myocardial infarction in a pati nt with sub arachnoid haemorrhage whose heart at autopsy was found to be normal was this association considered significant Subsequently Burch et al (4) reported that proloneed QT intervals large ab- normal T waves such as are seen in early myo- cardial ischaemia also occurred in addition to subarachnoid haemorrhage in patients with cer ebral haemorrhage and in a group of unclassi fied cerebrovascular accidents Prominent U waves were also observed in these conditions

In contrast to the increased T wave amplitude (4 5) large inverted T waves accompanying pro- longed QT and prominent U were reported in the same clinical groups (27) and attributed to cerebrovascular disease although eleven of the twelve patients in the series showed evidence of previous heart disease and the only one on whom autopsy was performed had myocardial infarc tion

Since then several papers mostly case reports have been published dealing with these abnormali ties (2 3 6 8 11 12 13 14 15 16 18 20 22 24 25)

In a series of 29 pati nts with subarachnoid haemorrhage (6) deep T wave inversions were observed in 15 prolonged T interval in 14 and an abnormally large U wave in eight patients This pattern of wide inverted T wave large in crease in the QT and a prominent U wave were describ d as pathognomonic by Hug nholz (13) and similar patterns were reported also by others (2 12 15 20)

However attention was also focused on the depression of the S-T interval as the most com mon abnormality occurring in 12 patients of 17 with intracranial bleeding (11) This abnormality has occasionally b en observed by others (8) Large abnormal T waves originally d scrib d as typical in cerebrovascular accidents (4 5) have also been occasionally observed since by other authors (24 25) Srivastava et al (25) observed the most marked S-T elevations in leads V2–V5 preceding T inversions which followed later

In contrast to most authors Shuster (24) pointed out the frequency of bradycardia and short QT Right and left bundle bran h block have been ob- served occasionally In only one case of sub arachnoid haemorrhage reported in the literature (22) were the ECG changes consistent with trans mural anterior myocardial infarction and the heart was found to be normal on autopsy

The few materials that have been published show considerable variation in the amount of

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Table I *Patiens with cerebrovascular accidents followed up by serial ECG tracings*

	No of pats	Excluded	Included	Age (range)	Years (mean)	Sex ♂	♀	Abnormalities in ECG (no of cases)	Per cent of included cases
Subarachnoid haemorrhage	48	13	35 (73)	15-6)	44 1	21	14	25	71 5
Intracerebral haemorrhage	48	41	7 (14 5)	25-61	45 7	4	3	4	57 1
Unclassified cerebro vascular accident	6?	45	17 (27 4)	40-72	56 1	14	3	7	41 1
Total	158	99	59			39	20	36	

ECG changes recorded in cerebrovascular acci dents

Bergstrom (2) observed T wave inversion and QT prolongation in three of 33 patients with various cerebrovascular disorders In a material of 44 patients from China (16) seven with sub arachnoid haemorrhages prominent U waves were observed in 33 prolonged QT in 32 and large inverted T in two cases

On the other hand in a series of 418 patients with various cerebrovascular disorders presented by Lichtlen and Schaub (18) only 13 or 3 1% showed ECG changes This is in striking con trast to the findings of Fentz and Gormsen (8) who reported an incidence of 71 % (15 out of 21) in intracranial bleedings and 15 % (11 out of 69) in cerebral infarctions

Marked differences exist in individual ECG changes Their frequency in cerebrovascular ac cidents and their origin has been a matter of controversy and speculation The present series has been analysed in order to throw more light on this important practical problem

MATERIAL AND OBSERVATIONS

The series consists of patients treated for cerebrovascular accidents in the Department of Medicine University of Oulu from Feb 1 1964 to Feb 1 1967 Of the 158 cases adequately covered by serial ECG recordings a large part were excluded because of previous hyper tension coronary disease or medical history of some cardiac diseas or such evidence observed on autopsy Cases suspected of pre-existing cardiac disease were also excluded from the material

The whole series of 158 cas s is presented in Tab e I according to the type of cerebrovascular accident

The great majority of patients with subarachnoid haemorrhage had no pre-existing cardiovascular disease and were included This is in striking contrast to the pa tients with intracerebral haemorrhage or unclassified cere brovascular accidents (mainly cerebral infarction)

In the group of subarachnoid haemorrhage 25 out of 35 patients (71 5%) showed ECG abnormalities The figures for patients with intracerebral haemorrhage or unclassified cerebrovascular accidents were 57 1 and 41 1 respectively but it should be noted that the num ber of cases in the group of intracerebral haemorrhage is relatively small

The individual ECG chang s in patients with subarach noid haemorrhage are presented in Table II

Of the total of 25 cases with abnormal ECG 11 showed abnormally large U waves TU fusion waves oc curred in six patients Prolongation of QT interval 20 % or more above normal (corrected for age sex and heart rate) was observed in eight patients S T segment eleva tion, most marked in chest leads V1-V4 was observed in six patients This abnormality only occurred in pa tients admitted to hospital very shortly after the cere brovascular accident, usually in connection with marked acute elevation of blood pressure as seen in Fig 1

This is also seen in a patient indicated by two asterisks in Table II whose serial ECG tracings are presented in *Fig 2*

The patient was admitted to hospital Feb 6 1965 with symptoms of vertigo and headache Ten minutes af ter admission she suddenly lost consciousness Lumbar puncture revealed bloody liquor and ECG showed eleva tions of S T intervals in leads V1 through V4 Blood pressure taken simultaneously was 230/150 mm Hg

The patient was treated according to principles in cur rent practice and her clinical condition improved The pattern of S T elevations was followed by deep T in versions seen in leads I AVL V2-V6 as shown in ECG recorded Feb 8 1965 After a nearly complete recovery she again complained of acute headache and went into coma on Feb 15 Blood pressure showed an acute in crease from 140/80-160/90 to 220/130 mm Hg during the attack ECG now revealed a pattern near y identical to those on admission with S T elevations in the chest leads (*Fig 2*) Feb 1? 1965 Clinical improvement was observed for several days and ECG again revealed the deep T wave inversions in leads I AVL, V? V6 with marked QT prolongation as before (*Fig 2* Feb 23 1965)

On Feb 27 the patient again became unconscious and

Table II *ECG changes in patients with subarachnoid haemorrhage*

Pat.	Age	Sex	Large upright T wave	Prominent U wave	Prolongation of Q T interval (% above normal)	Elevation of S-T segment	TU fusion	T wave inversion	QRS changes	Acute increase in B P ad mmHg	Clinical or autopsy findings
R T	34	♀		U							Clinical diagnosis with bloody liquor Angiography normal
P N	37	♂		U							Angiography aneurysma aortae communicans anterior
K M	43	♀			23	V1–V4				240 150	No preexisting hypertension Bradycardia Autopsy coronary arteries patent. Petechial haemorrhages under left ventricular endocardium mainly at the septum
K H	53	♀	II III AVF		33			I AVL V[illegible]–V5			Clinical diagnosis with liquor Angiography normal
K V	40	♂				V1–V4				200 100	Angiography aneurysma art. cerebri anterior l.dx
H I	56	♀		U	60*	V1–V4		I AVL V2–V6		230 150	Autopsy aneurysma art cerebri med l sin Coronary arteries were patent. Small yellowish confluent spots at the left ventricular side of the septum
H A	24	♀		U	[illegible]2						Angiography arterio-venous *aneurysm in left parasagittal* parietal region
A A	68	♀			31			AVL, V2–V4			Clinical diagnosis with bloody liquor
L O	41	♂	V2–V4				TU			200 120	Angiography aneurysma arteriae communicans posterior
L S	24	M		U				V5–V6			Angiography aneurysma arteriae communicans anterior
N E	27	♂		U		V1–V4				220 1[illegible]0	Angiography aneurysma arteriae cerebri media l.dx No preexisting hypertension
L E	35	♀			32*			V1–V4		190 110	Autopsy aneurysma arteriae cerebri media l dx. coronary arteries patent Small confluent haemorrhage spots under left ventricular endocardium. Histology myocardial infarction
V K	68	♂		U				V2 V6			Clinical diagnosis with bloody liquor
H L	41						TU				Clinical diagnosis with bloody liquor Angiography normal
H E	37	♀						V1–V4		[illegible]00 105	Autopsy aneurysma arteriae cerebri anterior l sin No cardiac pathology No preexisting hypertension
P P	36	♂		U							Autopsy aneurysma arteriae communicans anterior No cardiac pathology
M J	32	♂					TU	I AVL, V5–V6			Angiography aneurysma arteriae cerebri posterior l sin
M E	15	♀					TU				Clinical diagnosis with bloody liquor Angiography normal
I I	44	♂	V2 V4	U							Clinical diagnosis with bloody liquor No angiography
S A	9				37	V1 V3	TU	I AVL, V2 V6	QS V1 V3	[illegible]00 110	Clinical diagnosis with bloody liquor Angiography aneurysma arteriae communicans anterior

Table II (*cont*)

Pat	Age	Sex	Large upright T wave	Prominent U wave	Prolongation of Q T interval (above normal)	Elevation of S T segment	TU fusion	T wave inversion	QRS changes	Acute increase in B P ad mmHg	Clinical or autopsy findings
K V	40	♂		U							Autopsy aneurysma arteriae cerebri anterior l sin No cardiac pathology
K M	42	♂					TU				Autopsy aneurysma arteriae communi ans anterior No cardiac pathology
K E	23	♂		U				II III AVF			Clinical diagnosis with bloody liquor Angiography normal
A T	44	♂			20	V1–V4					An iography aneurysma arteriae cerebri mediae l dx
M E	69	♀	V2–V4							230/120	Clinical diagnosis with bloody liquor No angiography

Case histories of patients indicated with asterisks reviewed in brief in text

died a few hours later Autopsy revealed patent coronary arteries and the endocardium of the left ventricle showed yellowish confluent spots mainly on the left of the septum and papillary musc es A ruptured an urysm was located in the right middle cerebral artery

T wave inversions usually in *I AVL* and s veral chest leads were observed in ten patients In cas s where initial ECG changes were S T elevations T inversions also developed later in the leads reflecting the septal and anterolateral aspects of the heart In only one case with vertical rotation of the heart did thes chang s occur in leads II III AVF Abnormally large T waves or a high peaked T as seen in Fig 3 were obs rved in four patients

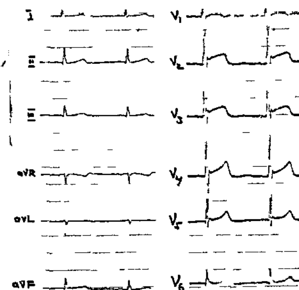


Fig 1 K V a *40* year-old man with subarachnoid haemorrhage indicated by one asterisk in Table II One hour after ons t of symptoms his blood pressure was 200/100 mm Hg and the ECG revealed bradycardia 44 min and marked elevations of S T s gments in leads V1–V4

In one case a *35* year old woman (indicated by four asterisks in Table II) with ECG changes of marked QT prolongation and T inversions in leads V1–V4 (Fig 4) autopsy showed myocardial infarction

This patient was admitted with symptoms of subarachnoid haemorrhage and ECG revealed the pattern of QT prolongation and T inversions in leads V1–V4 After nearly complete recovery acute exacerbation occurred two weeks after admission The patient died two days later Autopsy revealed massive haemorrhage in the right hemisphere and ruptured aneurysm was located in the right middle cerebral artery The coronary arteries were patent and small confluent haemorrhagic spots were s en under the left ventricular endocardium Histological examination revealed a small area where the myocardial fibres were fragmented had lost their nuclei and striations and were infiltrated with leucocytes The changes observed corresponded to the time interval from relaps to death

In this material one patient with subarachnoid haemorrhage (indicated by five asterisks in Table II) showed QRS changes (Fig 5)

This patient was a 29 year-old previously healthy woman who was admitted to the hospital for sudden loss of consciousness on Feb 4 1966 Her blood pressure was 200/110 Heart sounds were normal and there were no murmurs The heart was arrhythmic and ECG revealed ventricular ectopic beats of multifocal origin, QS complex through *leads V1–V3 and T wave inversions* in leads I AVL, V2–V6 The lumbar puncture taken despite ECG changes revealed bloody spinal fluid A subarachnoid haemorrhage was diagnosed The patient recovered and on Feb 11 the ECG showed T inversions in lead III, diminution of R in leads V–V4 elevated ST seg

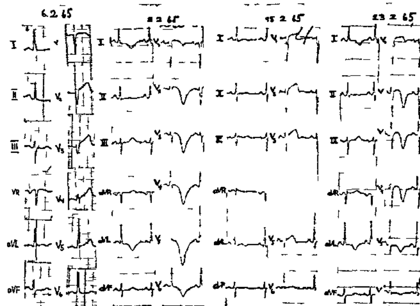


Fig The ECG of a patient with subarachnoid haemorrhage showing S T segment elevations in leads V1–V4 during acute increase in blood pressure (Feb 6 1965 and relapse Feb 15 1965) In the recovery phas these changes were followed by deep T inversions in leads I AVL, V1–V6 in normotensive state See text

ments and TU fusions in the same leads Angiography performed later revealed an aneurysm in an anterior communicating artery The patient made a complete recovery

In the group of intracerebral haemorrhage as shown in Table I, four of the seven cases showed ECG abnormalities There was a U wave in four T wave inver

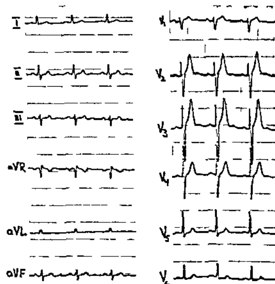


Fig 3 High peaked T waves in precordial leads V -V4 in electrocardiogram of a 41 year-old man with subarachnoid haemorrhage A small U wave is also visible in leads V2–V4 Serum electrolytes were normal

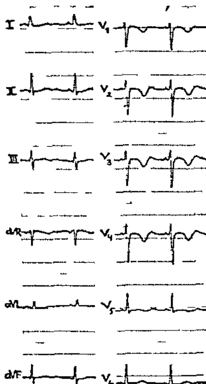


Fig 4 The ECG of a 35 year-old woman with subarachnoid haemorrhage showing QT prolongation and T wave inversion Autopsy revealed confluent haemorrhagic spots under left endocardium and histology revealed myocardial infarction The coronary arteries were patent

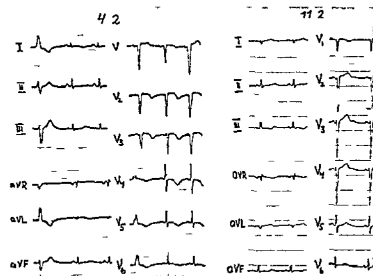


Fig 5 The ECG of a 29 year-old woman with subarachnoid haemorrhage showin ventricular ectopic beats QS complex through leads V1-V3 and T wave inversions in leads I AVL, V2-V6 on admission to hospital After clinical improvement the ECG revealed T inversion in lead III diminution of R in leads V2-V4 S T segment elevations and TU fusions in the same leads

sions in chest leads in three QT prolongation in two and S T segment elevation through V1-V3 in one patient Qualitatively the changes did not differ from thos observed in subarachnoid haemorrhage

In the group of unclassified cerebrovascular acciden s (mainly cerebral infarction) seven patients out of 17 showed electrocardiographic abnormalities (41 1) The U wave was the only abnormality in five patients prolonged QT and slight T inversions in precordial leads in two As a rule the ECG changes were markedly milder than in the group of intracranial haemorrhage In none of the present patients was hypokalaemia obs rved despite several tests

DISCUSSION

The few materials published in the literature show considerable variation as regards the amount of ECG changes associated with cerebrovascular accidents While Lichtlen and Schaub (18) in a series of 418 patients with various cerebrovascular accidents observed ECG changes in only 13 patients (3 1 %) Fentz and Gormsen (8) reported an incidence of 71 % in intracranial bleedings and 15% in cerebral infarction Other authors quote figures between these extremes (2 4 6 11 16) The incidence of ECG changes in the present material was 71 5 % in the group of subarachnoid haemorrhage and 57 1 % for intracerebral haemorrhages the latter group was however very small The majority of patients with unclassified cerebrovascular accidents had a prominent U wave as the only abnormality Only two out of 17 of the included patients (11 8 %) showed other abnormalities in addition to the U wave The figures for the present series therefore were very similar to those reported by Fentz and Gormsen (8)

While most authors (2 6 13 16 27) have been unable to confirm the existence of large abnormal T waves (4 5) but have described the pattern of prolonged QT deep inversions of T waves and prominent U waves as pathognomonic the present authors observed this to occur in four cases in the early phase after cerebrovascular accident Also marked S T elevations were observed to precede in many cases the T wave inversions which followed later In several patients this was shown to occur in connection with acute arterial pressure rise In none of the patients admitted several hours after the accident were large or high peaked T waves or S T segment elevations seen

The causes of these ECG abnormalities have been a matter of controversy and speculation Autopsy data have been available in very few of the reported cases and mostly revealed normal hearts (4 6 13 17 24) The conclusion has been drawn that these changes may be of cerebral origin and thus only simulate myocardial damage

Hypokalaemia (11) is however unlikely to be the causative factor as is indicated by electrolyte determinations (9 16 18 27) In none of the present patients was hypokalaemia observed

It has been shown that fluctuating potassium and calcium concentrations may lead to QT and T wave changes in the isolated heart (26) Although no significant extracellular disturbances could be demonstrated in the present material it

is nevertheless possible that there may be intra cellular alterations of electrolytes sufficient to alter the transmembrane potential

Burch et al (4) considered that the nature of ECG changes suggests sympathetic storms re sulting from the cerebral injury It has also been shown that infusions of epinephrine and norepi nephrine into a coronary artery lead to QT and T wave changes (1)

In contrast it has also been suggested that the ECG changes may be caused by lesions of area 13 on the orbital surface of the frontal lobe where the cortical representation of the vagus nerve is situated (6 10)

In experimental work on cats it has been de monstrated (19) that stimulation of discrete hypothalamic areas leads to temporary and reproducible pressor responses with a highly elevated blood pressure associated with consistent ECG changes similar to ischaemia Moreover intense or prolonged stimulation enhances the develop ment and persistence of ECG changes and in duces significant postmortem changes in th form of small haemorrhages and histologically distinct myocardial infarction mostly in the interventric ular muscle mass The pressor responses are completely abolished by C2 spinal section indica tive of sympathetic origin

Watts and co-workers (28) have demonstrated the effect of lesions of the hypothalamus on the gastrointestinal tract and heart in monkeys While gastric mucosal haemorrhages in connection with intracranial bleedings have been extensively re ported surprisingly few reports have be n pub lished on subendocardial haemorrhage

Koskelo et al (15) reported three cases of subarachnoid haemorrhage with ECG changes all of which showed subendocardial haemorrhagic spots mainly at the septum Similar subendocardial haemorrhages were seen in the present material and in one case a distinct myocardial infarction was observed

It has been shown that ECG changes are produced by various methods of causing cerebral injury (7 21 23) Eichbaum et al (7) under ex perimental conditions using a number of differ ent methods caused elevation of intracranial pressure in 80 mongrel dogs The brain injury and/or extradural compression led to a sharp in rease in blood pressure and to ECG changes In the initial period of several minutes the T waves were large or high peaked before reverting to characteristic negativity Diminution or even abolition of R together with deep Q waves in precordial leads completed the picture of recent myocardial infarction

The present authors find that the observations on their series strongly support the view that these same mechanisms are involved in clinical conditions

It must be stressed that very different forms of cerebral injury have similar effects on the ECG Evidently these injuries affect the brain as a whole leading to an intense increase of intra cranial pressure and it seems certain that the site of the primary lesion is of no significance The intense increase in intracranial pressure forces the cerebral mass into the direction of least resistance within the closed cranial box i e the foramen occipitale magnum This explains why the first symptoms of intracranial hypertension are respira tory arrest and elevation of blood pressure due to a direct or indirect impairment of hypothalamic centres (7)

The sudden rise in intracranial pressure re sults in bradycardia and acute increase of blood pressure as observed in several of the present cases followed by a sudden rise in left ventricular pressure and anoxia of the subendocardial layers of this ventricle This also explains why the ECG changes are much fewer and milder in cerebral infarction than in intracerebral bleedings

According to observations based on the present material the authors cannot overstress the state ment by Koskelo et al (15) according to which ECG changes in cerebrovascular accidents do not simulate myocardial ischaemia or injury but are signs of them It should however be borne in mind that th se lesions may be biochemical ana tomical or both

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THE SIGNIFICANCE OF THE STOMACH FOR THE ANTIPERNICIOUS PRINCIPLE IN THE LIVER OF PIGS IV

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Abstract Two patients with pernicious anaemia gave a mild but unmistakable response to injection of a liver extract Hepsol 4–64 from a pig in which the fundus was preserved after removal of the cardia and pylorus and which had been followed for 13 / months This is then another demonstration of the decisive aetiological importance of the fundal region to the antipernicious principle of the liver in pigs The still unknown causal relationship is discussed The hypothesis is advanced that the fundus of the pig plays a role in the utilization of the intrinsic factor (in the pylorus and duodenum)

In young animals (puppies pigs) total gastrectomy constantly gives rise to a clinically and morphologically well-defined chronic fatal symptom complex (experimental endogenous gastroprival p l lagra and most recently the experimental gastroprival symptom complex) (9 10 11 12 13 14)

The constant changes are arrest of growth emaciation changes in the skin and hair in the blood and bone marrow osteoporosis complete loss of antipernicious principle in the liver and severe degenerations in the central nervous system

By removing certain mucosal areas of the stomach (simple or combined elective resections) the aetiological role of these areas in the various components of the symptom complex has been studied (the experimental direct aetiological method" (18))

The present publication presents further investigations into the relationship between the fundus and one of the components of the gastroprival symptom complex viz. the antipernicious principle of the liver in the pig

PREVIOUS INVESTIGATIONS

Total gastrectomy on pigs completely abolishes the antipernicious effe t of the liver (15) This finding confirmed the studies of Bence (1) and of Goodman et al (4)

That operative removal of the fundus (total resection of the fundus) constantly causes the same disappearance of the liver effect as does total gastrectomy was first demonstrated by Petri et al in 1941 (15 16) The liver effect is greatly reduced at the end of about five months and completely lost in eight months At the time of operation the pigs were six weeks old

Borch Madsen and Søeborg Ohlsen in 1948 (2) reported to have found some liver effect in totally fundus resected pigs As the observation periods were 5 $^3/_4$ 6 $^1/$ and 9 $^1/$ months the postoperative disappearance of the liver effect is not yet complete at least not in the first two experimental pigs Incidentally the authors mention only two tests for the presence of liver effect without stating from which of the three operated pigs the preparations were derived

PRESENT INVESTIGATIONS

The object of the present resection experiment was to ascertain whether preserving the fundus alone can maintain the hepatic content of antipernicious principle Thus it is a counter-experiment to previous resections in which only the fundus was removed (17)

The experiment forms part of parallel studie on the aetiological significance of the gastric region to the gastroprival changes in the central nervous system The latter and the systemic sequelae of the operation for the experimental animal will be reported in a future paper (19)

Expe imental an mal

Combined elective resection of the cardia and pylorus, leaving the fundus, was done on a pig, Danish land race (No 149) The observation period before sacrifice was 13 / months

It should be emphasized particularly that the experimental animal had not shown signs of anaemia but a

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THE SIGNIFICANCE OF THE STOMACH FOR THE ANTIPERNICIOUS PRINCIPLE IN THE LIVER OF PIGS V

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Abstract Vitamin B_6—unlike nicotinic acid—cannot restore the antipernicious principle of the liver which is invariably lost after resection of the gastric fundus in pigs

The specific antipernicious principle of the liver is lost after total gastrectomy or resection of the fundus This loss has so far been compensated for in only one case i e in a fundus resected pig treated parenterally with nicotinic acid (1 2) From further experiments it was apparent that the cardia and not the pylorus was the important factor (3)

Vitamin B_6—like nicotinic acid—has been rather widely used in our experimental studies on the cause of the symptom complex resulting from the two types of operation

Therefore it seemed of interest to investigate whether vitamin B_6 also possessed the ability to restore the liver effect after it had primarily disappeared due to resection of the fundus

PRESENT INVESTIGATIONS

The experimental conditions were in principle the same as in the nicotinic acid experiment

A pig Danish land race (no 165) aged six weeks was subjected to elective resection of the fundus leaving the cardia as well as the pylorus After the gastroprival symptom complex had been allowed to run its course with the usual clinical characteristics, for nine months the pig was treated parenterally with vitamin B during the subsequent four months Thereafter the pig was killed The B preparation used was pyridoxine hydrochloride (Benadon Roche) The dosage was 50 mg e ery 3 days As the p g lost weight during the experiment from 50 to 35 kg the (large) weekly dose corresponded to 2.0–? 85 mg/kg body weight.

An extract from the li er of the experimental pig, Hepsol 5–37 was made in the usual way (1 3) and its possible antipernicious effect studied on two patients (women aged 59 and 73) suffering from typical pernicious anaemia Treatment The experimental preparation was injected i m in a dose of 10 ml, on two consecutive days After an interval of 13 and 10 days respectively the control preparation (Hepsol MCO) was administered in the same way One of the patients was later switched over to further treatment with other preparations

CASE REPORTS

Case 1

H O H a female aged 59 admitted to Medical Department III Kommunehospitalet, Copenhagen on 18 8 1945 and discharged on 25 9 During the past ten years she had been treated for hyperchromic anaemia. She was achlorhydric (Congo phenolphthalein 0/2 (normal 20-60/30-70)) her temperature was normal her tongue smooth and she had mild paraesthesiae Hb 65% R.B C 2.4 mill colour index 1 4 W B C 3360 (lymhpocytes 24%) Reticulocytes 0 7% Diameter of red cells 7.55 μ Anisopoikilocytos s with numerous microcytes and megalocytes.

Treatment 1 Hepsol 5–37 10 ml i.m daily for two consecutive days (22.8 and 23 8) ? interval of 13 days 3 Hepsol MCO 10 ml i m. daily for two consecutive days 4 followed for 14 days

Case 2

L. A. L. a female aged 73 admitted to Medical Department VII Kommunehospitalet Copenhagen on 18 6 1945 and discharged on 18 8 Untreated Achlorhydric (Congo-phenolphthalein 0/15) Temperature normal tongue smooth severe paraesthesiae of the limbs Hb 46 R.B C 1.8 mill colour index 1 2, W B C 3100 (lymphocytes 57%) Reticulocytes 0 9 Anisopoikilomacrocytosis. Sternal bone marrow (22.6) pernicious anaemia

Treatment 1 Hepsol 5–37 10 ml i.m daily for two consecutive days (6 6 and 27 6) 2 interval of ten days 3 Hepsol MCO 10 ml i m da ly for two consecutive days (8 7 and 9 7) 4 interval of 13 days 5 Hepsol high potency (MCO) 5 ml and iron tablets 23 7 and 30 7 6 Exopylorin two capsules daily from 1 8 7 Hepsol high potency MCO 5 ml on 8 8

Recapitulation

Liver extract from the experimental animal Hepsol 5–37 did not induce any increase in the

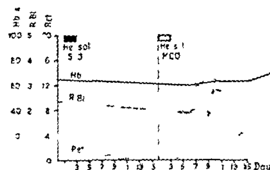


Fig 1 Case 1 No reaction in a pernicious anaemia patient on liver extract, Hepsol 5-37 from a pig submitted to resection of the gastric fundus and treated with vitamin B_4. Immediately afterward a normal reaction to the control preparation Hepsol MCO

reticulocyte count Hb level or R.B.C either in case 1 or 2 Subsequent administration of the control preparation Hepsol MCO induced in both patients a typical haematopoietic response (Figs 1 and 2)

DISCUSSION

Two patients with pernicious anaemia did *not* respond to liver extract from a fundus resected pig which had been treated parenterally with vitamin B_4 during the past four months out of a total observation period of 13 months

Thus vitamin B_4 could *not* restore the antipernicious effect of the liver which is invariably lost after resection of the gastric fundus

This result is at variance with the compensation for the loss of liver effect obtained by nicotinic acid in a previous experiment using the same experimental conditions Therefore pending further studies this effect of nicotinic acid must be considered an isolated phenomenon which so far remains unelucidated

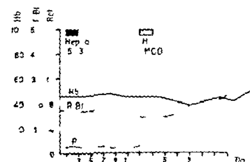


Fig 2 Case 2. Same absence of reaction as in case 1 in another pernicious anaemia patient on Hepsol 5-37 Immediately afterwards a normal reaction to Hepsol MCO

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EXTENSIVE MUSCLE NECROSIS AFTER LONG TERM TREATMENT WITH AMINOCAPROIC ACID (EACA) IN A CASE OF HEREDITARY PERIODIC EDEMA

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Abstract A 31 year-old man with hereditary periodic edema was treated with aminocaproic acid 30 g daily in two periods After respectively five and seven weeks of treatment he complained of pain in the thighs and calves The cell enzymes GOT GPT and CPK increased to high levels in the serum Myoglobin and an increased concentration of creatine were demonstrable in the urine Microscopic examination of the material from a muscle biopsy showed Zenker s hyaline degeneration of the muscle cells

The patient regained muscle strength rapidly after the first incidence but slowly after the second

He has now been free from episodes of edema for eight months on methyltestosterone therapy

Hereditary periodic edema or hereditary angioneurotic edema (HANE) is an unusual disease There is evidence that it is inherited as a mendelian dominant It is characterized by episodes of circumscribed non inflammatory edema in various parts of the body especially in the subcutaneous tissue and in the larynx In the latter event there is a big risk of asphyxia with high mortality Edema in the gastrointestinal tract with severe abdominal pains and in the cerebrum with various nervous symptoms is also common The edema appears regularly at intervals of from one week to one year (For a more extensive description of this disease see earlier publications (21 31))

Many theories have been evolved concerning the cause of the hereditary periodic edema (31) Not until the last 5–10 years however have fundamental discoveries been made to elucidate the nature of the disease In 1962 Landerman et al found that patients with HANE have a lowered activity of a plasma inhibitor against the kallikrein kinin system (22) Donaldson et al showed in 1963 that these patients lack also a plasma inhibitor against C'1 esterase which is an enzyme in the complement system (11) In the same year Kagan and Becker suggested that this inhibitor against C'1 esterase might equally be an inhibitor against kallikrein (19) Rosen et al in 1965 claimed that there are two genetic variants in hereditary periodic edema They found that one group of patients lack the C 1 esterase inhibitor whereas another group has an abnormal unfunctional inhibitor (32) Arnoldsson et al and Granerus et al in 1967 showed that even the histamine metabolism is changed in these patients (5 12)

Allergy has not been shown to play any part in the cause of the edema and anti allergic therapy and special diets have had no effect Various other drugs have also proved ineffectual for the treatment of this disease (21) Methyltestosterone therapy however has been used with success in a family of four members (35)

EACA a substance known to have inhibitory activity on several enzymatic reactions has also been used in the treatment of this disease Landerman in 1960 gave a patient with HANE 10 g of EACA intravenously during one attack of abdominal pain and during two attacks of subcutaneous edema However no definite clinical improvement could be observed (21) In three cases known to us EACA has been given orally with surprisingly good effect over a period of one to several years (13 23 26) In none of these patients have muscle symptoms been observed

In the present communication a patient with hereditary periodic edema is described who in

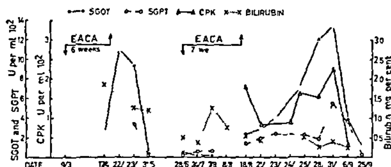


Fig 1 SGOT SGPT CPK and bilirubin in serum during and after two periods of treatment with aminocaproic acid in a patient with hereditary periodic edema

connexion with EACA treatment got extensive muscle necrosis

METHODS

Bleeding time wa performed by a modification of the method of Ivy et al (17)

Creatine in serum was determined according to Technicon Autoanalyzer file N 11 b using the Jaffé reaction

Creatine kinase (CPK) in serum wa determined according to Tanzer (36)

Factor V was determined a described by Aas (1)

Factor VIII The method of Hardisty and McPherson was used

Fibrinogen The method of Blomback (8)

Glutamic oxaloacetic transaminase (SGOT) in serum was determined according to Babson (6) modified for the Technicon Autoanalyzer system.

Glutamic pyruvate transaminase (SGPT) in serum was ined according to Henry (16) modified for the Technicon Autoanalyzer system

Haptoglobin in serum was determined according to Jayle (18)

Partial thromboplastin time (PTT) was performed as described by Waaler (37)

Plasminogen The method of Berg Korsan Bengtsen and Ygge (7)

Platelet adhesiveness The method of Hellem for determination of platelet adhesiveness in whole citrated blood was used (15)

Platelets were counted by the method of Brecher and Cronkite (9)

PP according to Owren and Aas (28)

Recalcification of citrated sil cone treated plasma Blood was collected with minimum stasis and with a clean puncture using coarse silicone treated needles and mixed with 1/10 of its volume of 3 citrate

All equipment was silicone-treated After centrifugation for 15 minutes at 1900 g, the plasma was collected Recalcification was performed with 0 2 ml plasma + 0 2 ml 0 05 *M* $CaCl_2$

Spectrophotometric analysis wa made with a Zeiss model M4 Q11 instrum nt.

Spontaneous fibrinolytic activity was determined on fibrin plates made from plasminogen free fibrinogen and thrombin 0 1 ml of an euglobulin precipitated at pH 6 4 was applicated on the plate

CASE REPORT

Male age 31 years From the age of seven he has had episodes of abdominal pain and sudden appearance of edema in his face and on his hands and feet. During childhood he was admitted to hospital several times on account of abdominal distress At the age of ten he was operated on for a ductus arteriosus persistens Botalli At the age of fifteen he was investigated several times for an obscure jaundice finally interpreted as a juvenile familiar non hemolytic jaundice In the beginning of 1960 he was admitted to hospital several times for circumscribed edema urticaria and obscure abdominal pain

The episodes of peripheral edema have persisted In recent years cerebro lesional symptoms and mental depre sion have appeared

A thorough investigation of the family history reveals *that his mother grandfather on his mother s side* two of his mother s sisters and a son of one of them, have since their youth complained of sudden appearances of circumscribed edema similar to those that trouble our patient Moreover his mother on one occasion had a severe attack of hemolytic anaemia

In 1965 when the patient was first admitted to the Department of Internal Medicine II Sahlgrenska Hospital, Göteborg, no explanation of the patient s complaints was found. Routine laboratory examinations revealed no abnormal values except a slight hyperbilirubinemia, at most 2 8 mg per 100 ml serum of which 59% was of the indirect reacting type On this occasion an increased percentage of sideroblasts in the bone marrow (86%) was also observed

In February 1967 the activity of C 1 esterase inhibitor in serum was analysed An abnormally low activity of this inhibitor as well of C 4 and total C was found in the patient s serum and also in the sera of his mother one of his mother s sisters and her son (The analyses of

Table I *Results of the study on blood coagulation and fibrinolysis before and during the second period of muscle necrosis*

	Platelets	Bleeding time (min)	Platelet adhesiveness ( )	Partial thromboplastin time (sec)	Recalcification of citrated silicone treated plasma (min)	Factor VIII ( )	PP ( )	Factor V ( )	Plasminogen (units)	Spontaneous fibrinolytic activity (mm^2)	Fibrinogen ( )
14 10 66	154 000	3		58			130	150			0 24
2 6 67	182 000	5	60	51	4	180	115	145	15	121	0 24
18 8 67	165 000	14	49	68	10	160	110	140	8 2[a]	—	0 31
21 8 67	709 000	13		59	6	140	125	150	5 3[a]	—	0 ,8
Normal values	150–300 000	< 11	40–60	50–70	3–10	50–150	70–130	60–140	12 18	80–140	0 20–0 40

Probably because EACA was still present in the plasma

the e fa tors in the complement system were performed by Assistant Professor Anna Britta Laurell Institute of Medical Microb ology University of Lund Lund, Swe den.)

In March 1967 therapy with EACA was started in an oral dosage of 30 g daily Sl ght side-effects such as orthostatic reactions appeared early About five weeks later he first complained of pains in the thighs and calves, and found walking difficult. The SGOT concentration was found to be increased to 250 I U and serum bilirubin to 1 7 mg per 100 ml serum therefore EACA therapy was discontinued

Two days later the patient became worse He now complained of extreme exhaustion, increasing muscle pains and observed that his urine was brownish red On the evening before admission to hospital he became cyanot c for a short time The doctor on duty found him pal and very tired. However there were no signs of re piratory or circulatory insufficiency and he had no muscle tenderness An immediate analysis of the SGOT ga e a value of 1080 I U Unfortunately no analysis of myoglobin and hemoglobin in the urine was performed on this occasion Heparin and dicumarol were administered on the suspicion that dissem nated intravascular coagula tion might be the cause of the acute muscle symptoms. With this therapy he gradually improved and was almost free from symptoms after one week.

SGOT and SGPT concentrations were ele ated for about a week. Serum bilirubin concentrat on, howe er did not exceed 1 3 mg 100 ml serum (Fig. 1) Hemoglobin concentrat on was 15 4 g 100 ml blood white cell count 6000 per mm blood platelet count ___000 per mm and haptoglobin concentrat on 85 mg 100 ml serum ESR increased from _ to 15 mm h Thymol flocculation test, alkaline phosphatase activity and serum creatinine con centrat on were all with n normal limits

The patient was aga n admitted to hospital in late June 1967 for a new control. Data from this period are illustrated in Fig. 1 *Histological examination of muscle* biopsy material from m gastrocnemius showed normal muscle structure EMG from m.m. quadriceps dxt and sin. were also found normal.

Treatment with EACA, with the same dosage as before was started for a second time during this period in June 1967 Slight side-effects such as rhinitis edema in the face and orthostatic reactions appeared early A single high value of serum bilirubin was occasionally observed after about fi e weeks of treatment SGOT SGPT and CPK, however were all normal at this time Seven weeks after the start of the EACA treatment muscle pains ap peared especially in the proximal muscle groups of the extremities, but also in m trapezius and m rectus abdominis. The patient was now admitted to ho pital at once and EACA therapy was immediately discontinued Neither exhaustion nor cyanosis appeared on this oc casion. Remarkable increases in the SGOT SGPT and especially CPK in serum were found. The serum bilirubin concentration, however was w thin normal limits (Fig. 1) ESR now reached 40 mm per h Other routine laboratory tests were normal.

The urine was browni h red in color Spectrophotometric analys s of urine strongly suggested that the excreted pig ment was myoglobin Creatine excretion in urine was in creased to a maximum of 170 mg per day Porphyrine in urine was normal Histologi al examination of a muscle biopsy material from the right thigh now revealed very distinct focal changes in an otherwise normal muscle structure Single muscle cells showed hyaline degeneration with lost transverse striation swollen muscle mass chang ing in hyaline scales, breaking down to necrosis. In the muscle cells where the changes were most pronounced the scales of necrosis were surrounded by a thin sack of sarcolemma and endothelium l ke nucleus and containing a few macrophages and small round cells. Nothing re markable except stasis was observed in the small vessels The picture was that of Zenker s hyaline degeneration and necrosis. (The histological examination was performed by Professor Jan Mellgren Department of Pathology I Un *versity of Goteborg, Goteborg, Sweden) ECG and X ray* of the chest gave no indication of heart muscle damage

Blood clotting tests made on several occasions in 1966 1967 and 1968 were all normal before as well as during *the periods with muscle symptoms (Table I)*

The serum enzyme activities gradually became normal as seen in Fig 1 At this time the patient regained muscle strength very slowly and he was not free from muscle symptoms until two months later EMG from m quadriceps in November 1967 however still showed slight changes of myopathia

During the two periods of EACA treatment the patient observed a decreasing tendency to edema Abortive episodes did appear but at longer intervals than before treatment.

From November 1967 methyltestosterone linguets (Perandren T) 10 mg three times daily has been administered On this therapy the patient ha now been free from episodes of edema for eight months

DISCUSSION

Nausea diarrhea and dizziness in standing position are side-effects of aminocaproic acid treatment that have often been reported (3 4)

Thromboembolic complications after short term EACA treatment have been discussed in the literature and a few instances of this complication have been reported (3 25 26) The correlation between EACA therapy and thromboembolism is still not established

In one instance of bleeding from a prostatic carcinoma treated with EACA for two days in a total dose of 26 g necrotic changes were found in the liver and in the heart muscle at necropsy one month later the author could not explain (27) Death resulted from a cerebral hemorrhage

During long term treatment with EACA no severe side-effects have been reported in man In one study seventeen patients with hemophilia A were treated with 138–1200 mg EACA/kg/day for 2–16 months One patient complained of nausea No other symptoms of toxicity were reported Periodic complete blood counts urine analysis and hepatic function studies on this group of patients showed no abnormal findings (30) In another material thirty two patients with progressive systemic sclerosis were treated with 16–32 g EACA/day for periods up to 36 months The authors concluded that toxic reactions to the drug were not severe and were reversible (33 34)

In animal studies EACA has been given to rats in a dose of 500–5000 mg/kg/day for three months without any toxic damage being seen in the liver heart lungs or kidneys (24)

In dogs and monkeys treated with high doses of EACA intravenously for three weeks however subendocardial hemorrhages focal thickening of the subendocardium and myocardial fatty degeneration have been observed (10) In an autopsy material of forty six patients who died from their disease soon after treatment with EACA no subendocardial bleeding was observed and none of the organs showed any sign of toxic effects of the drug (26)

In the case reported in this paper extensive muscle necrosis occurred on two occasions Both episodes were preceded by five to seven weeks of daily treatment with high doses of EACA The patient had never before had muscle symptoms The connexion between the muscle necrosis and the treatment with EACA therefore seems reasonably established

The changes in the muscle tissue are those of Zenker s hyaline degeneration This special form of degeneration has been described in severe infectious diseases such as typhus diphteria and Weil s disease and also in anaphylactic shock The cause of Zenker s degeneration has been thought to be ischemic The following two hypotheses will therefore be discussed

Because EACA is an inhibitor of fibrinolysis the possibility of intravascular coagulation with the formation of microthrombi must be taken into account It is improbable that the administration of EACA alone can be responsible for extended intravascular coagulation However in our patient an increased level of serum bilirubin was observed on several occasions from childhood up to the present The bilirubin was mainly of the indirect reacting type indicating an increased hemolysis The patient s mother who also lacks C 1 esterase inhibitor has been treated in the hospital for a severe episode of hemolysis It is not clear whether this symptom is related to their inborn defect In our patient hemolysis seemingly appears periodically as does his edema There is no correlation in time however between the two phenomena Judged from the relatively small increases in bilirubin concentrations and the fact that the hemoglobin values have been normal at every control the hemolysis must be of a minor degree

Hemolysis of red blood corpuscles may cause thrombosis (20) probably because red cells release clot promoting substances (29) In our patient no

symptoms that could be related to thrombi have ever appeared in periods with a raised serum bili rubin value However it was thought that if a hemolytic episode occurred when the fibrinolytic activity was blocked by EACA a thrombotic pro cess might have been initiated This hypothesis would explain the lag of six weeks between the start of the treatment and the first symptoms of the muscle disease

Against this hypothesis stand the facts that no thrombi were observed in the small vessels in the biopsy material and no consumption of platelets or clotting factors were seen in connexion with the episode of extended muscle necrosis As will be seen from the figure the increase in serum bilirubin did not coincide with the muscle symp toms The hypothesis of intravascular coagulation is thus not supported by the observations How ever it cannot be excluded It is a well known fact that thrombi often are not demonstrable in ca es of intravascular coagulation

The other hypothesis to be discussed is that the known effect of EACA to release noradrenalin from the nerve endings (2) could perhaps cause ischemic muscle necrosis The release of nor adrenalin secondarily to EACA treatment how ever is of a rather short duration and is followed by a decreased release (2) The consequences for the circulation are a vasoconstriction followed by a vasodilation Nothing is known of the effect of long term administration of EACA on the nor adrenalin release However there will most prob ably be an exhaustion of noradrenalin in the nerve endings and thus a vasodilation The ortho static reaction reported as a side-effect is prob ably explained by this mechanism Any connexion between the muscle necrosis observed in our pa tient and the effect of EACA on the noradrenalin release from the nerve endings seems improbable

Thus we cannot explain the mechanism under lying the muscle necrosis appearing after EACA treatment in our patient It is possible that in addition to the innate defect referred to the patient has enzyme defects involving the muscle cells A study is planned to investigate this aspect Moreover as EACA treatment of HANE has produced encouraging results and will probably be used to treat other patients in the future we find it of value to report this serious side-effect in our patient

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THE INFLUENCE OF PHENOBARBITAL ON THE HALF LIFE OF DIPHENYLHYDANTOIN IN MAN

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Abstract Prior treatment of human subjects with phenobarbital for ten days increased the rate of disappearance of an intravenous dose of [14]C labelled diphenylhydantoin in 1 patients The phenobarbital treatment did not change the amount of ultrafiltrable diphenylhydantoin nor the volume of distribution of diphenylhydantoin and it is therefore considered justifiable to assume that phenobarbital may increase the metabolism of diphenylhydantoin in humans In two patients who were intoxicated with barbiturates the half life of diphenylhydantoin was estimated to 4 25 and 3 75 hours, respectively which are very low compared to the normal value of 11 3 hours

It has been demonstrated in animal experiments (4) that the half life in the blood of a number of drugs e g. phenylbutazone and aminopyrine is shortened during the administration of phenobarbital In vitro experiments using rat liver microsomes have shown that this is due to more rapid metabolism of the drugs concerned Phenobarbital seems to increase the amount of drug metabolizing enzymes in the liver The phenomenon is termed enzyme induction

Dayton et al (7) have shown that administration of phenobarbital to patients who are receiving dicoumarol therapy leads to a reduction in the dicoumarol concentration in the blood with a simultaneous decrease in prothrombin time Other observations have shown that the concentration of griseofulvin in the blood is reduced after the administration of phenobarbital (2) It is reasonable to assume that the effect of phenobarbital in man is also due to enzyme induction but as yet there is no direct evidence of this

Cucinell et al (6) have found that the half life of diphenylhydantoin in the blood is also considerably reduced after the administration of phenobarbital to rats and dogs After giving phenobarbital to five epileptic patients who had been treated with diphenylhydantoin 300 mg per day for at least fourteen days the same authors (5) observed a fall in the blood concentration of diphenylhydantoin Kutt et al (8) have carried out a similar trial However they found no difference between the serum diphenylhydantoin values in two groups of epileptic patients one of which had received both diphenylhydantoin and phenobarbital and the other diphenylhydantoin alone On giving phenobarbital to epileptic patients who had received diphenylhydantoin in a constant dosage for longer periods they found that the serum diphenylhydantoin level could fall rise or remain the same

As there is a discrepancy between these results we have found it of interest to determine the serum half life of diphenylhydantoin in a number of patients before and during the administration of phenobarbital

METHODS

The half life of diphenylhydantoin in the blood (T/2) was determined by following the radioactivity in the blood after i v injection of 100 mg diphenylhydantoin to which 15 μC 4 [C] labelled diphenylhydantoin had been added The samples of serum were taken approx 2, 5 8 and 12 h after the injection. The diphenylhydantoin was extracted as follows Five ml of serum was transferred to a glass stoppered test tube containing 12.5 ml borate buffer (pH 9 0) and 16 ml of *n* heptane *n*-butanol (3 3 1) The mixture was shaken for 5 min and centrifuged for a further 5 min. The upper layer was transferred to another test tube containing 12.5 ml of the borate buffer shaken for 5 min and centrifuged. This upper layer was transferred to a test tube containing 15 ml 0 5 *N* NaOH shaken and centrifuged The upper layer thus obtained was discarded and 14 ml of the lower layer was transferred to a new test tube containing 5 ml . *N* HCl + 15 ml chloroform The test tube was shaken and centrifuged

Table I *The first and the second half life and volume of distribution for diphenylhydantoin in the control group and the phenobarbital group*

The last column indicates the time interval between the two determinations

Pat	T/2 (h) 1st	T/2 (h) 2nd	Volume of distribution (l) 1st	Volume of distribution (l) 2nd	Time interval (days)
Control group					
O J	6 50	6 25	33 6	38 4	14
H L.	10 50	14 75	47 5	50 0	14
E B	9 25	8 16	49 1	40 7	12
S L	8 [illegible]5	6 50	53 4	41 8	12
E N	11 00	10 00	34 4	37 2	13
N D	7 75	8 75	54 8	47 3	12
E N	20 25	2[illegible] 00	—	—	14
P R	7 00	8 50	48 0	44 2	6
E E	11 50	9 75	40 1	36 8	13
A N	10 00	8 00	43 8	46 1	13
Mean	10 2	10 3	44 9	42 5	12 3
Phenobarbital group					
N L.	15 00	10 00	50 8	43 6	9
A H	6 75	7 00	48 4	33 8	20
L. J	6 92	6 67	58 1	46 7	8
M P	11 00	11 25	4[illegible] 6	43 9	10
S H	6 66	5 00	34 6	29 4	7
A C	8 00	7 00	52.4	45 7	11
K P	9 16	8 75	—	—	7
E. H	15 00	10 66	—	—	8
T L.	13 00	11 75	31 4	35 2	9
A M	10 75	8 00	34 00	37 2	8
I R	8 00	6 75	33 [illegible]	36 4	10
A H	15 25	7 25	66 8	59 6	10
Mean	12 49	8 48	45 2	39 4	9 8

again and a 125 ml aliquot of the chloroform layer was evaporated to dryness under a flow of nitrogen The residue was redissolved in 250 μl of absolute alcohol 100 μl of the alcohol extract was taken for liquid scintillation counting (liquid scintillation fluid consisted of 0 15 g POPOP + 3 5 g PPO + 50 g naphthalene dissolved in 500 ml of dioxane) The counts were plotted against time on a semilogarithmic paper and from the straight line obtained the T ½ was calculated Another 100 μl of the above mentioned alcohol extract was used for thin layer chromatography (silica gel ether chloroform (15 85)) to check that the radioactivity corresponded only to the region of phenylhydantoin on the chromatogram

The phenobarbital in the serum was determined by a method described by Lous (9)

The distribution volume of diphenylhydantoin was determined as the ratio between the total counts of labelled diphenylhydantoin injected and the counts in the serum sample at zero time which was found by extrapolation

Ultrafiltration was employed for the determination of the free non protein bound diphenylhydantoin 1 66 mg diphenylhydantoin labelled with ^{14}C was added to 100 ml serum and allowed to equilibrate for 1–2 h the serum was ultrafiltered at room temperature at pH 7 6 for about 20 min with the formation of about 300 μl ultrafiltrate The ratio between the activity in the ultrafiltrate and that in serum was taken as an expression of the free non protein-bound diphenylhydantoin

None of the subjects investigated showed any evidence of hepatic or renal disease as judged from serum bilirubin GO transaminases, alkaline phosphatases and serum creatinine levels.

RESULTS

For the assessment of the normal values we have determined the half life of diphenylhydantoin by the procedure described above in 29 patients between the ages of 18 and 78 years T/2 varied between 6 6 h and 26 7 h with an average of 11 3 h This wide variation in half life from one individual to the next is in accordance with the findings for other drugs e g phenylbutazone (1)

In ten patients T/2 was determined twice at an average interval of 12 3 days at the same times the apparent volume of distribution was determined (Table I control group) During the interval none of the patients had received drugs which might have influenced T/2

In 12 volunteers T/2 was determined before and during treatment with phenobarbital in daily doses of 1 5 to 2 5 mg per kg body weight. This resulted in serum phenobarbital values between 1 1 and 4 0 mg per 100 ml The time for phenobarbital treatment averaged 9 8 days The results are shown in Table I phenobarbital group together with the apparent volume of distribution.

In the group without phenobarbital treatment (control group) the mean initial T/2 was 10 2 h and in the group with phenobarbital treatment 12 5 h In the latter group there is a decrease in T/2 during phenobarbital treatment to 8 5 h which is significant $0\ 02 < p < 0\ 05$ It is seen that in the five humans with the greatest decrease in T/2 the mean initial T/2 was 16 5 h and in the rest of the group 8 85 h

We had the opportunity to determine T/2 in two patients who were intoxicated with pheno-

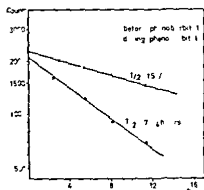


Fig. 1. The disappearance rate of ^{14}C-labelled diphenylhydantoin in blood from one subject before and during phenobarbital treatment.

barbital T/2 was 4 25 and 3 75 h respectively Both patients suffered from chronic abuse of phenobarbital and the serum concentration of phenobarbital was 12 6 and 8 8 mg per 100 ml, respectively at the time of T/2 determination Both patients were awake

Three patients were given 200 mg phenobarbital perorally 12 h prior to the *determination* of T/2 In two of them T/2 was unchanged whilst in the third T/2 decreased by 25 per cent.

There was no significant change in the volume of distribution of diphenylhydantoin between the first and second T/2 determinations in the two groups (Table I)

The in vitro addition of phenobarbital to serum in therapeutic concentrations (1 3–3 3 mg p r 100 ml) did not affect the proportion of the ultra filtrable diphenylhydantoin which was found to be 6–7 per cent of the total amount

Fig. 1 shows an example of the half life graph in one subject Before the administration of pheno barbital T/2 was found to be 15 25 h whilst after ten days of treatment it was 7 25 h

In all the patients chromatography of plasma samples revealed that these contained only di phenylhydantoin

DISCUSSION

In a group of 12 volunteers there was a significant fall in the T 2 in blood after ten days of phenobarbital treatment this was not found in a control group consisting of ten persons who received no phenobarbital treatment The groups seem reasonably comparable concerning the initial T/2 and the time interval between the two determinations It is not possible from the present study to estimate what duration of phenobarbital treatment is neces sary in order to reduce T/2 in only one out of three patients was there a fall of 25 per cent after a single dose of phenobarbital 12 h prior to the determination of T/2

The very low T/2 values 4 25 and 3 75 h re spectively in two patients during phenobarbital intoxication add further evidence to the suggestion that phenobarbital treatment may decrease T/2

Our results are in accordance with the findings of Cucinell et al (5) who in five patients found a decrease in the concentration of diphenyl hydantoin after adding phenobarbital Although the T/2 for the group as a whole decreased sig nificantly some of the patients showed no de crease of T/2 during phenobarbital treatment, and this is in part in accordance with the results reported by Kutt et al (8) who found that the concentration of diphenylhydantoin decreased in one third and remained in the same range in one third of patients however it offers no explana tion of the fact that in the remaining third of their patients there was a rise in the concentration of diphenylhydantoin after addition of phenobarbital

Cucinell et al (5) found that the decrease in diphenylhydantoin concentration was particularly marked in individuals with high initial levels of diphenylhydantoin In the five of our patients who showed the greatest decrease in T/2 the mean initial T/2 was 16 5 h compared with 8 85 for the rest of the group

According to Butler (3) diphenylhydantoin is oxidized in the liver to parahydroxyphenylhydan toin and excreted as such in glucurinated form in the urine Our chromatographic studies show that the blood contains only unchanged diphenylhydan toin both before and during phenobarbital treat ment

We found that about 93 per cent of the di phenylhydantoin in blood is bound to plasma proteins and as stated by Martin (10) the half life in the plasma of drugs with high degree of protein binding is not a direct expression of the rate of elimination of the drug from the whole body We have however found no change in the amount of free ultrafiltrable diphenylhydantoin after addition of phenobarbital and similarly no significant change in the volume of distribution of diphenylhydantoin during phenobarbital treat

ment It is consequently reasonable to assume that the change in the half life in blood is an expression of a more rapid elimination of diphenylhydantoin from the whole body This may possibly in analogy with the findings in the in vitro experiments using animal liver microsomes be due to an increased rate of conversion of diphenylhydantoin to para hydroxyphenylhydantoin in the human liver

The wide individual variations in T/2 from 11 3 to 26 7 h in 29 patients are an important factor in explaining why it sometimes is difficult to predict the dosage needed to obtain a desired diphenyl hydantoin concentration in the blood in some epileptics

The practical aspects of this investigation again emphasize the necessity of checking the plasma concentration of diphenylhydantoin in epileptics at regular intervals

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PLASMA FREE FATTY ACID TURNOVER RATE IN OBESITY

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Abstract Free fatty acid turnover rate was measured by constant infusion of 1 C palmitic acid complexed to human albumin in eight extremely obese patients and in seven controls after a 12 hour fast In some of these patients these measurements were performed also during work in a few of the obese patients before and after physical training The results were expressed in relation to different body compartments It was found that free fatty acid turnover rate was higher at rest in the obese than in controls This difference disappeared when free fatty acid turnover rate was calculated per kg body weight or per kg body fat When calculated per kg lean body mass however the obese patients again had higher values of free fatty acid turnover rate than the controls During a standard work load the obese did not show lower free fatty acid turnover rates during work than the controls even if the increase in two of the obese patients who had a high turnover rate at rest was small during work The results thus give no evidence of a decreased fatty acid turnover rate in obesity On the contrary after fasting for 12 hours it seems to be higher than normal

The metabolism of the enlarged adipose tissue in obesity has attracted much interest not least concerning the mobilization of fat During fasting the free fatty acids (FFA) of plasma originate mainly from adipose tissue and consequently the plasma FFA have been studied repeatedly in obesity

Dole (10) found fasting venous levels of FFA in obese patients apparently proportional to the degree of obesity Gordon (15) later confirmed the high FFA levels in obesity Klein et al (20) on the other hand found an inverse correlation between FFA in plasma and body size in a material which included no extremely obese patients Gordon (15) reported a delay in FFA increase during prolonged fasting This was later confirmed by Opie and Walfish (25) and was interpreted as a deficient FFA mobilization in obesity

Measurements of FFA after different stimuli for their mobilization other than fasting have also been repeatedly made in obesity Epinephrine injections have been tried (26) as well as exposure to cold (13) and in a few investigations the effect of exercise on FFA has been studied in obesity as a more physiological stimulus to adipose tissue mobilization Opie and Walfish (25) found a diminished response of FFA in plasma after a short period of exercise Klein et al (20) observed a damped FFA curve in obese patients during exercise similar to that of patients given glucose Issekutz et al (18) however recently found no evidence of a decreased increment of FFA concentration in obese patients during work

Several factors complicate the interpretation of adipose tissue lipid mobilization from the FFA concentration in plasma First this level is dependent not only on the production of FFA from depots but also on the outflow of FFA to consuming tissues Second the FFA production from adipose tissue is the resultant of lipolysis and reesterification and these two processes are regulated by different mechanisms which to a large extent are independent of each other Third interpretations of hormonal and substrate controls of adipose tissue metabolism are easier when the amount of active tissue taking part in lipid mobilization is known viz the size of adipose tissue depot and its cellularity

Attempts have been made to circumvene these difficulties by studies of adipose tissue in vitro Such studies have shown that lipid mobilization is decreased in obesity per unit of adipose tissue weight while fatty acid outflow and lipolysis per adipose tissue cell are not abnormal (6) If one assumes an increased number of fat cells in the obese patients studied and the in vitro results are extrapolated to the situation in vivo this would

Table III *FFA turnover rates in relation to different body compartments in controls and obese patients*

	FFA turnover rate (μEq/min)	FFA turnover rate/kg body weight (μEq/min)	FFA turnover rate/kg fat (μEq/min)	FFA turnover rate/kg lean body mass (μEq/min)
Control	505±76	6.9±1.0	6.7±3.6	9.5±1.5
Obese patients	994±101	9.2±1.0	?.9± 8	15.7±1.6
	P 0.01	Not significant	Not significant	P<0.0

min (women) during 30 min sitting on an ergometer bicycle. Blood samples were taken at the 5th, 10th, 20th and 30th min of work and then at 5, 10, 20 and 30 min after work with the patient again sitting in a chair.

FFA turnover rate was calculated as described by Havel et al. (16). Three of the obese patients (B.B., I.G., M.W.) were subjected to physical training by a procedure described previously (?7) and were thereafter reexamined in a similar way.

Body fat and lean body mass were calculated from weight (W) and height (H) utilizing the regression equation (0.18 (W/H × 10³) − 23.?) obtained by Edwards and Whyte (11) by comparison between W/H and body compartment determinations with antipyrin space. In some instances determinations of body compartments were also performed with the aid of total exchangeable potassium and total body water according to the techniques and calculations utilized by Moore et al. (?4) as modified by Lindholm (1?).

RESULTS

Table II presents the results of FFA turnover as a mean of the three samples at rest and the mean of the two last samples during work. FFA concentration as well as turnover rate of FFA at rest was significantly higher in the obese patients than in controls. The oxygen uptake during work did not show much difference between the obese and the controls. As a matter of fact the averages are very similar, 1556 and 1529. During this work load the FFA turnover was apparently not lower in the obese patients than in the controls. The increase from resting values was limited in the obese patients who had a high FFA turnover rate at rest (B.B. and I.G.). Physical training in these two patients lowered the lactate concentration during work, but did not increase FFA turnover rate during work. Patient no. 3 (M.W.) showed no decrease in lactate after training.

Table III gives the results of resting FFA turnover rate determinations in relation to different body compartments. It is seen that even if the total turnover rate of FFA is increased in the obese patients, the turnover rate of FFA per kg body weight is not increased. Nor is it increased when calculated per kg body fat. Per kg lean body mass, however, the obese patients showed a significant increase in FFA turnover rate.

DISCUSSION

It has been shown both in the dog and in non-obese man (17) that the plasma FFA concentration correlates well with FFA turnover rate. Plasma FFA concentration is the result of an FFA inflow rate from adipose tissue and an outflow rate to fatty acid consuming tissues. Wide individual variations in the quantitative relationship between these two types of tissues may be suspected to influence the correlation between FFA concentration and FFA turnover rate. This has also been demonstrated by Issekutz et al. (17) who have shown that at the same plasma FFA concentration obese patients had a higher turnover rate than normal subjects, and the latter higher than dogs, presumably with less adipose tissue than humans. Therefore it is not directly possible to interpret plasma FFA concentration in obesity as a measure of lipid mobilization in comparisons with non-obese controls.

The material of obese patients consisted mainly of women while the controls were exclusively men. In vitro adipose tissue from both sexes does not show any differences in lipid mobilization (8). It seems unlikely that this difference in composition of the two materials should be of any significance.

The present work thus shows that FFA turnover rate at rest is increased in obesity. This has recently also been found by Issekutz et al. (17). When expressed per kg body weight, however, there was no difference between the plasma FFA turnover rate of obese and controls.

Calculations of body compartment weights ac-

cording to the regression equations of Edwards and Whyte (11) are subject to uncertainties as pointed out by these authors The results of these calculations were checked in some patients in the present work by determinations of total body fat with the aid of total body water and exchange able potassium (21 22 24) and were in these cases found to agree fairly well Until more reliable determinations are available these results have to be interpreted as preliminary They appear to show that the FFA turnover rate is the same p r kg adipose tissue weight in obese patients and in controls Per kg lean body mass however the turnover rate of FFA seems to be increased in the obese cases

These results then suggest that lipid mobiliza tion in obesity is not decreased during fasting On the contrary it is higher than in non-obese humans The FFA consuming tissues presumably comprising the main part of the lean body mass are thus furnished with a surplus of FFA The present study also suggests that FFA turnover rate per kg adipose tissue is similar in obesity and in controls Obese patients usually have fewer fat cells per unit weight of adipose tissue than con trols (7) Taken together this indicates that when FFA turnover rate is calculated per fat cell there does not seem to be a deficient lipid mobilization in obesity either This is in general agreement with findings in vitro (6) but this question needs further penetration before more quantitative conclusions can be drawn.

When measurements were performed during work the patients were in circulatory steady state FFA fluxes were also reasonably stable but the lactate concentration was usually in a decreasing phase

Previous studies of FFA mobilization in obesity during work have been performed on the basis of FFA conc ntrations and not with measurements of FFA turnover rate (18 20 25) The difference in correlation between FFA concentration and turn over rate between obese and controls has been discussed above Furthermore during work frac tional turnover rate of FFA increases abruptly (9) producing a decrease in FFA concentration The FFA concentration may therefore be misleading as an index of FFA mobilization during work and comparisons between obese patients and controls may be difficult

The present investigations during exercise are few and allow only limited conclusions These ex periments do not indicate a decreased lipid mobilization in the obese patients during exercise since the FFA turnover rates were apparently not lower in the obese during a standard work load

The increment of FFA turnover rate during work was small in two obese patients (B B and I G) who showed high resting FFA turnover rate values Inhibition by lactate of FFA turnover rate increase during work might be discussed since both lactate infusions (19) and lactate in in vitro systems (4) inhibit lipid mobilization This explanation is not likely however since these two patients did not increase their FFA turnover rate during the same work load after physical training when the lactate increase was smaller

In conclusion this work has given no evidence to indicate a decreased lipid mobilization in obesity at rest or during work On the contrary at rest lipid mobilization seems to be increased perhaps also when expressed per unit lean body mass FFA turnover rate per kilogram body weight or adipose tissue did not seem different in obese patients and controls The conclusions including measurements of body compartments must so far be considered as only preliminary Whether the increase in FFA turnover rate in obese patients is due to an increased lipolysis or a deficient FFA reesterification in adipose tissue is the subject for current studies

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EFFECTS OF HIGH PROTEIN AND LOW PROTEIN DIETS ON ORNITHINE CARBAMOYL TRANSFERASE ACTIVITY IN HUMAN SERUM (S OCT)

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Abst act A protein-rich diet (proteins accounting for 84% of the calories) for one week resulted in a con tinuous rise of S-OCT in seven healthy subjects, the avera e level at the end of the week being almost twice that at the start. Thi d et was then replaced by a normal diet, whereupon the S-OCT level rose to three times the initial level and then declined continuously No significan change in S-OCT was noted when a protein free diet was gi en for one week. S-GOT and S-GPT rose moderately when the supply of proteins was interrupted. The find ings suggest that conditions whi h involve an increased protein br akdown result in elevated S-OCT leve s, prob ab y due to an increased synthesis of OCT in the liver

The enzyme OCT which is involved in the production of urea o curs in mammals almost ex clusively in the liver As a rule liver injury is ac companied by a rise in S-OCT (16) and this rise has been held to be a sensitive and specific sign of such injury (11 16) Elevated S-OCT levels are also found, however in conditions which presumably do not involve any real liver injury e g 4-7 days after operation trauma (3) Since these rises in S-OCT occur in connection with catabolic states with an increased production of urea it is conceivable that an augm nted protein breakdown per se may elicit the rise in S-OCT via an increased synthesis of the enzyme in the liver This conception is supported for instance by Schimke s (21) experiments on the rat, in which all conditions involving an increased breakdown of tissues also resulted in an increased content of OCT in the liver If a connection does exist be tween increased protein breakdown and a rise in S-OCT such a rise should also follow an increased exogenous administration of amino acids The present study was undertaken in order to investi gate whether S-OCT levels in healthy subjects are elevated via an increased load on the urea cycle enzymes when the supply of protein in the diet is augmented

MATERIAL

The study was made on seven healthy subjects, none of whom had any known history of liver disease All subjects had normal le els for S-OCT S-GPT and S-GOT at the start of the experiments, and their bilirubin values were less than 0 5 mg 100 ml. The subjects ae s x, height and weight are given in Table I.

Experimental procedure

The experiment lasted 25 days, subd vided into four pe riods as follows

Period 1

Protein week. Duration on week. The subjects ate a standard diet that varied according to the ind vidual s size and age Subjects 1 . and 5 were to eat 2400 cal., subjects 3 6 and 7 2100 cal and subject 4 1800 cal., 84 being in the form of protein. The subjects were not always able to consume all of the larg vo umes in volved but they never left more than 5% of a food portion un aten. Blood samples were taken for enzyme analysis initially as well as after 1 ., 4 and 7 days on the protein diet. Urine samples were collected during the 24 hours before each blood sample was taken The sampled urine was acidulated w th concen ra ed acetic acid.

Examples of diets veal, ham, chicken hare pheasant, hazel-hen, reindeer partridge white of egg, lobster crab shrimps, p ke burbot, cod turbot, gelatin

Period

Duration one week. The subjects received a standard normal diet ad libitum. Blood and urine were sampled at the same times and in the same way as in period 1

Period 3

Carbohydrate week. Duration one week. The subjects received a standard diet that aried according to the in

Table I *Age sex height and weight in 7 subjects on high protein and low protein diets*

Case no	Sex	Age (y)	Height (cm)	Weight (kg)
1	♂	[illegible]1	189.5	69.0
2	♂	22	181.0	68.5
3	♀	[illegible]8	164.0	50.0
4	[illegible]	52	161.0	48.0
5	♂	22	175.5	63.0
6	♀	[illegible]8	164.0	57.0
7	♂	31	179.5	71.5

dividual's size and age Subjects 1 2 and 5 were to eat [illegible]400 cal subjects 3 6 and 7 2100 cal., and subject 4 1800 cal carbohydrates accounting for 90 and protein for 5 The subjects had no difficulty in eating the whole of the stipulated portions Blood and urine samples as for period 1

Examples of diets rice potatoes, macaroni beetroot carrots parsnips artichokes turnips rusks, crispbread white bread ryemeal porridge potato-flour onion soup bilberry soup prunes cornflakes raisins apples, grapes pears lingonberry jam raspberry jam marmelade sugar treacle honey

Period 4

Duration four days The subjects received a standard normal diet ad libitum Blood and urine were sampled in the same way as in period 1 after 1 2 and 4 days

The subjects became very tired and irritable during the protein week (period 1) The voluminous diet made mealtimes troublesome The subjects never felt hungry Some of them had gastrointestinal complaints such as diarrhoea or constipation They drank fluids copiously during this week. In the carbohydrate week (period 3) the subjects had no difficulty in eating the stipulated portions They occasionally felt hungry but not unduly so

The protein and carbohydrate diets were supplied by Klingstaskolan (School of Domestic Science) Danderyd Sweden

METHODS

Recognized methods were used for the statistical calculations (6 23) The reproducibility (coefficient of variation) of a method was calculated from duplicate determinations expressed as a percentage of the mean of these determinations

$$\frac{100\sqrt{\frac{\Sigma d^2}{2n}}}{\bar{x}}$$

The OCT activity was determined by incubation of serum with citrulline carbamoyl ^{14}C in arsenate buffer (17) The results are expressed in nanomoles (nm) CO_2 liberated by 0.5 ml serum during two hours incubation under standard conditions. Normal value < 4 nm (?) i.e 0.004 micromoles $^{14}CO_2$. Reproducibility 8%

GOT and GPT were determined by the NADH method (10 24) as modified by Ordell (15) Normal value < 40 Karmen units (1) Reproducibility 5

The total nitrogen in the urine was determined by Kjeldahl analyses according to the procedure described by Hiller Planzin & Van Slyke (9) Reproducibility 4%

The urea nitrogen in serum and urine was determined with an autoanalyzer (Technicon Auto Analyzer) according to a slightly modified version of the procedure described by Marsh et al (13) Reproducibility 5%

The creatinine was determined with an auto analyzer (Technicon Auto Analyzer) according to the procedure of Hawk et al (8) which is a modification of the procedure of Folin and Wu Reproducibility 5%

RESULTS

The average initial S OCT level was 0.7 nm The level rose continuously during the protein week reaching a value of 1.2 nm towards the end of the period This rise is statistically significant ($P < 0.01$) There was a further rise after the subjects had started eating the normal diet the average level being 2.0 nm both 1 and 2 days after

Table II *S OCT (nanomoles $^{14}CO_2$) in 7 subjects on high protein and low protein diets*

Case	Initial level	Days after start of experiment														
		Protein period				Normal food				Carbohydrate period				Normal food		
no	0	1	2	4	7	8	9	11	14	15	16	18	21	22	23	25
1	0.5	0.9	1.1	0.5	1.1	4.0	3.7	3.3	1.4	1.4	0.8	0.9	0.5	2.1	1.5	0.8
2	1.0	0.5	0.7	1.4	1.6	3.3	2.8	0.6	0.[illegible]	0.4	0.2	0.2	0.3	0.6	0.3	0.4
3	0.4	0.8	0.9	1.0	1.0	1.6	1.6	0.9	0.8	0.8	0.8	0.6	1.0	1.0	1.2	0.5
4	0.8	0.9	0.7	[illegible].0	1.6	1.6	1.5	1.0	0.7	0.8	1.1	1.1	0.7	0.8	1.[illegible]	1.0
5	0.5	0.5	0.4	0.8	0.9	1.0	1.[illegible]	0.9	0.7	0.6	0.4	0.2	0.6	0.7	0.7	0.5
6	0.4	0.5	0.6	0.6	0.8	1.0	1.4	1.3	0.4	0.3	0.5	0.3	0.4	0.6	0.5	0.5
7	1.2	1.4	1.6	1.1	1.4	1.6	1.8	1.4	1.5	1.3	0.9	1.0	1.2	0.8	1.1	1
Mean	0.7	0.8	0.9	1.1	1	[illegible].0	[illegible].0	1.3	0.8	0.8	0.7	0.6	0.7	0.9	0.9	0.7
S.D	0.3[illegible]	0.33	0.40	0.52	0.33	1.17	0.91	0.90	0.48	0.4[illegible]	0.31	0.39	0.41	0.53	0.43	0.31

the end of the protein week These increases are also statistically significant ($P < 0.02$ and < 0.01 respectively) S-OCT then fell continuously No significant change was observed during the carbohydrate week Cf Table II and Fig 1

S-GPT showed a significant rise one day after the end of the protein week ($P < 0.05$) and S GOT two days after the end of the protein week ($P < 0.05$) Cf Tables III–IV

Serum urea was significantly increased during the protein week ($P < 0.01$) Cf Table V

The average urinary excretion of nitrogen was 11 2 g before the experiment started It rose significantly during the protein week ($P < 0.001$) reaching a peak after four days (32 5 g) The amount of nitrogen in the urine fell continuously after the end of the protein week During the carbohydrate week it showed a significant fall ($P < 0.01$) the lowest value (5 5 g) being noted after seven days Cf Table VI and Fig 1

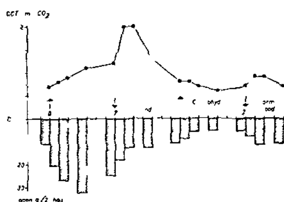


Fig 1 Variations in S-OCT and urinary excretion of nitrogen in seven subjects on high protein and low protein diets

Like total nitrogen the amount of urea in the urine rose significantly during the protein week and fell significantly during the carbohydrate

Table III *S GPT (Karmen units) in 7 subjects on high protein and low protein diets*

	Days after start of experiment															
Case	Initial level	Protein period				Normal food				Carbohydrate period				Normal food		
no	0	1	2	4	7	8	9	11	14	15	16	18	21	22	23	25
1	18	18	15	18	20	20	26	17	18	21	14	19	20	29	20	19
2	0	29	24	28	20	28	33	0	19	24	15	23	17	15	13	15
3	11	10	14	15	16	15	13	14	15	18	22	21	18	14	17	13
4	20	0	19	16	18	20	21	16	15	13	20	12	11	10	13	12
5	15	18	16	10	15	17	13	12	18	15	14	16	14	9	16	15
6	16	16	14	17	16	18	17	13	9	13	11	14	13	12	15	16
7	0	20	20	17	20	22	20	20	17	16	18	15	17	20	18	20
Mean	17	19	17	17	18	20	20	16	16	17	16	17	15	16	16	16
S D	3 4	5 7	3 7	5 8	2 2	4 2	7 2	3 2	3 4	4 1	3 9	4 0	3 4	7 0	2 6	2 9

Table IV *S GOT (Karmen units) in 7 subjects on high protein and low protein diets*

	Days after start of experiment															
Case	Initial level	Protein period				Normal food				Carbohydrate period				Normal food		
no	0	1	2	4	7	8	9	11	14	15	16	18	21	22	23	25
1	15	18	20	15	20	20	20	15	18	0	0	16	25	26	21	17
	15	18	19	16	15	27	24	15	16	15	20	15	15	0	12	0
3	10	16	14	13	1	14	16	15	12	20	20	27	22	13	16	15
4	29	28	33	23	0	4	9	20	15	18	18	20	15	11	13	0
5	16	16	14	17	18	23	20	17	16	17	17	14	20	15	20	18
6	14	15	13	15	19	15	15	14	17	15	15	13	15	9	0	11
7	18	0	20	17	14	18	0	0	18	19	17	16	15	20	15	16
Mean	17	18	19	17	17	0	21	17	16	18	18	17	18	16	17	17
S.D	5 9	5 0	6 9	3 2	3 2	4 8	4 8	2 5	2.1	[illegible]	2.0	4 8	[illegible]	6 0	3 6	3 1

Table V *Serum urea (mg urea N per 100 ml) in 7 subjects on a high protein diet*

Case no	Days after start of protein diet 0	1	2	4	7
1	17	25	27	37	3
2	12	25	23	27	24
3	14	23	26	31	28
4	12	21	23	23	20
5	16	26	29	35	33
6	14	24	23	28	29
7	17	28	35	34	34
Mean	15	25	27	31	29
S D	2 1	2 2	4 4	5 0	5 1

week ($P<0\ 001$) Cf Table VII There was a slight increase in urinary creatinine during the protein week ($P<0\ 05$) Cf Table VIII

DISCUSSION

The biosynthesis of urea takes place with the aid of the five urea-cycle enzymes OCT carbamoyl phosphate synthetase argininosuccinate synthetase argininosuccinase and arginase Arginase was the first of these enzymes for which an increase was demonstrated in water homogenates of rat liver after feeding a protein rich diet (12) It has since been shown (20 21) that feeding a protein rich diet to rats results in increased activities in liver homogenates for all five urea-cycle enzymes and that for two of them—OCT and arginase—the increase was due to increases in specific electrophoretically homogeneous protein S OCT was not investigated in these animals

Enzyme induction involving OCT in man does not appear to have been demonstrated A direct demonstration of such induction in the present study would have required liver biopsies which

Table VI *Urinary excretion of nitrogen (grams per 24 hours) in 7 subjects on high protein and low protein diets*

Case no	Days after start of experiment: Initial level 0	Protein period 1	2	4	7	Normal food 8	9	11	14	Carbohydrate period 15	16	18	21	Normal food 22	23	25
1	13 5	9 9	34 0	51	39 0	27 1	18 2	0 4	14 5	12 9	7 8	7 7	7 1	8 3	14 0	13 8
2	10 8	15 3	22 2	7 9	1 2				12 1	9 8	5 0	4 4	5 4			
3	14 6	3 0	26 3	35 8	25 6	22 9	14 4	18 2	13 5	10 0	[illegible]6	6 0	6 6	11 7	16 1	11 8
4	7 9	14 8	17 4	16 0	13 6	11 1	8 7	6 7	5 2	4 3	3 5	4 4	2 8	5 7	5 4	7 5
5	12 7	3	30 7	34 0	26 0	17 9	12 2	9 8	9 7	7 4	6 6	6 6	4 9	5 7	11 7	10 8
6	8 5	16 0	3	25 4	0 0	11 8	11 1	9 1	9 3	8 5	5 7	4 6	4 7	9 0	11 0	9 8
7	10 6	2 5	36 8	37 4	27 6	17 0	14 7	12 4	11 3	9 5	7 9	6 5	6 8	7 0	12	11 9
Mean	11 2	0 5	7 1	32 5	4 7	18 0	13 2	1 8	10 8	8 9	6 2	5 7	5 5	7 9	11 7	10 9
S D	2 5	5 5	7 0	11 0	7 9	6 2	3 3	5 4	3 1	6	1 6	1 3	1 5	2 3	3 6	2.1

Table VII *Urinary excretion of urea (grams urea N per 24 hours) in 7 subjects on high protein and low protein diets*

Case no	Days after start of experiment: Initial level 0	Protein period 1	2	4	7	Normal food 8	9	11	14	Carbohydrate period 15	16	18	21	Normal food 22	23	25
1	11 3	27 3	30 5	43 6	33 6	25 0	19 0	17 0	1 6	10 1	5 9	6 4	5 1	7	11 8	
2	7 0	13 7	20 0	24 7	18 4				10 2	7 9	5 2	3 6	3 5			
3	10 9	0 7	22 5	31 0	3 0	19 8	12 3	12 9	1 1	8 5	5 2	4 7	4 5	10 0	12 8	
4	5 9	12 2	15 5	13 5	10 7	8 6	6	5 3	4 6	3 1	3 5	9	1 6	4 0	4 2	
5	9 7	19 8	29 4	9 7	22 8	12 7	9 8	8 4	8 3	5 0	4 7	5 1	3 2	5 0	9 3	
6	5 9	13 2	20 7	22 6	17 3	9 6	8 9	6 7	8 0	7 1	4 5	3 4	3 9	7 0	9 5	
7	7 0	19 7	33 1	32 1	23 9	14 4	10 3	9 9	9 9	7 9	6 4	4 7	4 4	5 0	8	
Mean	8 2	18 1	24 5	28 2	21 4	15 0	11 1	10 0	9 4	7 1	5 1	4 4	3 7	6 4	9 4	
S D	2 3	5 4	6 5	9 3	7 1	6 3	4 4	4 3	2 5	3	1 0	1 2	1 1	2	3 0	

Table VIII *Urinary excretion of creatinine (grams per 24 hours) in 7 subjects on high protein and low protein diets*

Case no	Days after start of experiment																
	Initial level	Protein period				Normal food				Carbohydrate period				Normal food		
	0	1	2	4	7	8	9	11	14	15	16	18	21	22	23	25
1	2.19	2.36	2.27	3.40	2.24	1.9?	1.91	2.58	2.14	2.15	1.85	2.03	1.86	1.88	1.82	
2	1.68	0.93	1.84	1.50	1.92				1.83	1.79	1.8[illegible]	1.26	1.50			
3	1.1	1.45	1.45	[illegible].60	1.66	1.19	1.01	1.19	1.40	1.09	1.04	0.99	0.93	1.17	0.88	
4	1.05	1.23	1.12	1.04	1.12	1.04	1.08	0.74	0.75	0.69	0.88	0.92	0.69	0.84	0.69	
5	1.78	1.99	1.96	2.47	2.12	1.49	1.54	1.42	1.8	1.58	1.48	1.59	1.[illegible]7	1.05	1.65	
6	1.36	1.60	1.59	1.85	2.05	1.01	1.13	1.30	1.60	1.28	1.16	1.19	1.12	1.51	1.18	
7	1.75	[illegible].43	2.06	2.0[illegible]	1.87	1.41	1.38	1.48	1.98	1.58	1.71	1.33	1.53	1.60	1.61	
Mean	*1.56*	*1.71*	*1.76*	*2.13*	*1.85*	*1.34*	*1.34*	*1.45*	*1.65*	*1.45*	*1.42*	*1.33*	*1.27*	*1.34*	*1.31*	
S.D.	0.41	0.57	0.39	0.78	0.37	0.34	0.34	0.61	0.46	0.48	0.40	0.38	0.40	0.39	0.46	

were not practicable Since it is unlikely that the protein diet elicited any liver damage the rise in S-OCT in the present subjects was probably not ascribable to enzyme leakage as a result of changes in cell permeability Considering that S-OCT is held to be a specific sign of liver injury (11 16) it is remarkable that healthy subjects displayed a significant rise in S-OCT under physiological con ditions even though only two of the subjects reached the upper limit for normal S-OCT activ ity The rise in S-OCT is due either to an in creased flow of OCT from the liver or to a re duced elimination of OCT from plasma. OCT is reported to disappear from the serum also during biliary stasis and severe liver damage (16) The present healthy subjects displayed no signs of liver damage nor was there any reason to suspect damage to other organs that might have influenced elimination mechanisms The elevation of S-OCT activity was thus probably due to an increased outflow of the enzyme from the liver rather than to a reduced elimination from serum

An increased outflow of OCT from the liver might be due either to an increased synthesis of OCT or to permeability changes resulting in an increased escape of OCT from the liver cells If the synthesis of OCT was not increased however the continuous rise in S-OCT during the protein week must have involved a reduction of the liver s content of OCT This seems unlikely since it has been shown (19 20) that a protein rich diet in creases the content of OCT in the rat liver The total amount of OCT in the blood is very small compared with the total amount in the liver (16) making it difficult to assess the importance of moderate changes in S-OCT levels It does not seem particularly likely however that an in creased need of urea-cycle enzymes in healthy subjects would lead to such changes in the perme ability of liver cells that the outflow of OCT from these cells is facilitated The continuous rise in S-OCT activity during the protein week may therefore reflect an increase in the OCT activity in the liver as a result of an augmented synthesis Assuming that the turnover of OCT between liver and plasma remained unchanged (i e the same relative leakage during the experiment) the S-OCT levels indicate that the liver s content of OCT had almost doubled by the end of the protein week which is an elevation of the same magnitude as that observed after a protein rich diet had been given to rats (20)

S-OCT *tripled in the present subjects when the* supply of protein was suddenly reduced after a week This may have been because some of the enzyme that was no longer required was released from the liver cells The fact that the rise in S-OCT was not greater may indicate that the major part of the superfluous enzyme was broken down in the liver cells

Experiments using amino acids labelled with ¹⁵N have shown that amino acids are metabolized in the same way in the liver regardless of whether their source is exogenous or endogenous (22) which means that both exogenous and endogenous protein breakdown should result in an increased synthesis of OCT in the liver Experiments on the rat (21) suggest that this is the case The pre dominance of catabolic processes after operation trauma (14) is transformed into an anabolic pre

dominance after 4–7 days (14) which is also the time when there is nearly always a rise in S-OCT (3) In keeping with the line of reasoning above this elevation of S OCT seems to be open to the same interpretation as the rise after the protein week i e part of the OCT that was synthetised during the increased protein breakdown is probably released from the liver into the blood stream This theory is supported by the significant correlation that has been demonstrated between the postoperative excretion of nitrogen and the rise in S-OCT after 4–7 days (5) At the same time however some of the patients in the study cited displayed a tenfold rise in S-OCT but this was not the case with any of the present subjects Other factors may therefore be involved as well at least in some cases in the rise of S OCT 4–7 days after an operation It is possible that in these patients besides the increase in S OCT supposedly induced by increased protein breakdown other factors contribute e g an altered turnover of OCT between liver and plasma The importance of protein breakdown is indicated by the fact that other conditions involving an increased breakdown of body proteins—e g burn injuries (18) and radiation of malignant tumours (4)—also result in elevated S OCT levels after 4–7 days

The clinical relevance of these results seems to be that moderate increases of serum enzymes should be interpreted cautiously—they may reflect normal biological process Caution in this respect has also been recommended on clinical grounds (7)

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SERUM ORNITHINE CARBAMOYL TRANSFERASE ACTIVITY (S OCT) AFTER IRRADIATION OF MALIGNANT TUMOURS AND AFTER ADMINISTRATION OF ACTH

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Abstract A group of 20 women who received radiation therapy for cancer of the uterine cervix displayed a significant rise of S-OCT 4-6 days later This rise can hardly be explained by an increased adrenocortical stimulation elicited by the trauma because a corresponding stimulation (90 IU ACTH) in ten healthy subjects resulted in a significant drop in S-OCT A study of the nitrogen balance in six other subjects showed that adrenocortical stimulation with 90 IU ACTH did not lead to any increase in the breakdown of protein. These observations seem to support the theory that the increases in S OCT that were observed a few days after conditions involving an elevated breakdown of protein may be due to a release of some of the additional amount of OCT that is supposedly formed in the human liver during the maximal breakdown of protein

It has been found that operation trauma is followed after 4–7 days by a rise in S OCT which was assumed to be induced at least in part by the postoperative breakdown of protein (4) This rise in S-OCT however is significantly correlated to the postoperative increase in the excretion not only of nitrogen but also of 17 hydroxycorticosteroids (7) The present study was undertaken in order to elucidate the importance of protein catabolism and stimulation of the adrenal cortex for the rise in S OCT 4–7 days after operation trauma

MATERIAL, EXPERIMENTAL DESIGN

A In order to elicit a stimulation of the adrenal cortex comparable to that after operation trauma, ten healthy male subjects aged about 25 years were given 90 IU ACTH (Cortrophin Prolongatum®) intramuscularly once a day for three days Since it was difficult to control the subjects diet for practical reasons they were allowed to eat a normal diet ad libitum. Venous blood for the analysis of S OCT and serum urea was sampled before during and after the days when the ACTH was injected (Tables I-II)

B In order to investigate whether the above stimulation of the adrenal cortex in itself affects the excretion of nitrogen the same dose of ACTH (Cortrophin Prolongatum®) as under *A* above i e 90 IU a day for three days was given to a further six persons (cases 11-16) Of these cases 11 and 12 were patients with bronchial asthma case 13 was a patient with cerebral haemorrhage and cases 14 15 and 16 were normal healthy subjects The amount of protein in the daily diet corresponded to 10 g nitrogen All the urine produced in the 24 hours before and during the days when the injections were given was collected for the analysis of total nitrogen and urea nitrogen (Table III)

C In order to obtain a breakdown of tissue comparable to that after operation trauma a study was made of 20 women (cases 17-36) who received radiation therapy at Radiumhemmet for cancer of the uterine cervix. This therapy was given according to the present individualized Stockholm technique (16) and the study was conducted on the patients at the time of their first radiation session After the patient had been anaesthetized with halothane according to the principles described in a previous paper (4) radium inserts were applied into the uterine cavity and the vagina The radiation therapy lasted for about 20 hours The patients were in a good general condition both during and after the therapy with no clinical signs of circulatory failure or renal insufficiency Samples of venous blood were taken for the analyses of S OCT and serum urea before as well as 1 4 6 and 8 days after the start of radiation therapy (Tables IV–VI) Many of the patients were discharged after 3 or 4 days and the last blood samples were taken at the outpatient department. One woman who had pathologically high serum transaminase (>40 Karmen units) and S OCT >4 0 nm at the start of the study was excluded from the series

All subjects had been fasting for about 12 hours before the blood samples were drawn During the sampling period they refrained from physical activity and the consumption of alcohol

Table I *S OCT (nanomoles) in 10 subjects before and after the administration of 90 IU ACTH per day for 3 days*

Case	Initial level 0	Days after start of ACTH administration 1	2	3	4	5	7	9	11
1	1 4	0 6	0 9	0 8	0 2	0 1	0 7	0 3	0 6
2	1 7	0 8	1 1	0 4	0 8	0 9	1 2	1 5	0 8
3	0 3	0 2	0 3	0 7	1 0	0 7	1 [illegible]	1 2	1 4
4	1 [illegible]	0 9	0 4	0 5	0 5	1 2	0 8	0 5	1 2
5	0 6	1 0	0 7	0 5	0 9	0 9	0 9	0 7	0 7
6	2 7	1 0	1 1	0 4	1 5	2 8	1 6	1 4	1 6
7	1 3	1 4	1 6	1 0	1 1	1 2	1 0	1 6	2 1
8	0 4	0 2	0 3	1 5	0 3	0 4	0 6	0 0	0 6
9	0 7	0 4	0 3	0 5	0 7	0 9	0 8	0 6	1 1
10	1 5	0 5	0 1	0 3	0 6	0 3	1 3	1 8	1 4
Mean	1	0 7	0 7	0 7	0 8	0 9	1 0	1 0	1 [illegible]
S D	0 7	0 4	0 5	0 4	0 4	0 8	0 3	0 6	0 5
Range	0 3 2 7	0 2 1 4	0 1-1 6	0 3 1 5	0 2-1 5	0 1-2 8	0 6 1 6	0 0-1 8	0 6-[illegible] 1

METHODS

Recognized methods were us d for the s atistical calculations (9 [illegible]5)

The reproducibility (coefficient of variation) of a method was calculated from duplicate determinations ex pressed as a percentage of the mean of these determinations

$$\frac{100}{\bar{x}}\sqrt{\frac{\sum d^2}{2n}}$$

The OCT activity was determined by incubation of s rum with citrulline carbamoyl C in arsenate buffer (1) The results are expressed in nanomoles (nm) CO liberated by 0 5 ml serum during two hours incubation under standard conditions Normal value <4 nm (2) i e 0 004 micromoles CO Reproducibility 8%

The total nitrogen in the urine was determined by Kjeldahl analyses according to the procedure described by Hiller et al (11) Reproducibility 4

The urea nitrogen in serum and urine was determined with an autoanalyzer (Technicon Auto-Analyzer) according to a slightly modified version of the procedure described by Marsh et al (18) Reproducibility 5%

RESULTS

A The average level of S-OCT before stimulation of the adrenal cortex was 1 2 nm During

Table II *Urea nitrogen in serum (mg/100 ml) in 10 subjects before and after the administration of 90 IU ACTH per day for 3 days*

Case	Initial level 0	Days after start of ACTH administration 1		3	4	5	7	9	11
1	18	14	13	18	14	13	19	17	15
2	16	11	1[illegible]	11	10	14	13	16	18
3	22	19	16	15	15	18	21	0	17
4	[illegible]0	[illegible]0	1	1	17	19	19	22	19
5	16	13	14	16	17	16	14	17	15
6	24	[illegible]1	21	4	0	19	[illegible]0	2[illegible]	6
7	17	13	14	12	16	14	18	18	17
8	13	11	11	10	11	10	9	13	15
9	9	7	7	6	7	7	10	9	9
10	19	14	17	1	14	15	15	17	14
Mean	17	14	15	15	14	15	16	17	17
S D	4 3	4 4	4 4	5 4	3 8	3 9	4 2	4 0	4 3
Range	9 24	7 1	7 [illegible]1	6-24	7 0	7 19	9 21	9 [illegible]2	9 6

Table III *Excretion of total nitrogen and urea nitrogen in 6 subjects before and after the administration of 90 IU ACTH per day for 3 days*

Case	Sex	Age	Days after start of ACTH administration							
			0	1	2	3	0	1	-	3
			Total nitrogen (g/24 h)				*Urea nitrogen (g/24 h)*			
11	♂	45	12 0	1? 8	12 7	11 3	9 9	10 6	11 0	9 5
12	♀	37	10 1	12 0	11 7	12 1	7 0	9 3	8 4	8 7
13	♂	61	15 4	12.5	15 9	15 1	12.6	10 6	13 3	12 5
14	♀	22	8 3	8 5	8 5	8 7	6 2	6 8	6 7	6 8
15	♀	24	11 2	10 7	11 4	10 4	9 5	8 8	9 4	8 9
16	♂	33	12 2	12 1	11 4	11 7	10 3	10 0	9 0	9 8
Mean			11 5	11 4	11 9	11 6	9 3	9 4	9 6	9 4

stimulation S OCT declined to 0 7 nm this drop is statistically significant ($p < 0.05$) When stimulation ceased S-OCT gradually returned to the initial level (Table I)

The average amount of urea nitrogen in serum before stimulation of the adrenal cortex was 17 mg/100 ml 24 hours after starting ACTH stimulation the average amount was 14 mg and after 2 and 3 days stimulation it was 15 mg/100 ml these drops are statistically significant ($p < 0.01$) (Table II)

B No changes in relation to the initial level were found in the six subjects in whom total nitrogen and urea nitrogen were measured in the urine before and during stimulation of the adrenal cortex (Table III)

C The average S-OCT activity was 1 3 nm before radiation 2 2 nm on the 1st day after the start of radiation 4 6 nm on the 4th day 3 2 nm on the 6th day and 2 4 nm on the 8th day The increases on day 4 and day 6 are statistically significant ($p < 0.02$ and $p < 0.01$ respectively) (Table V)

Table IV *Age weight stage of cancer and dose of intracavitary radium in 20 women suffering from cancer of the uterine cervix*

Case	Age	Weight	Stage	Ra (mg h)
17	50	56 3	II B	2850
18	46	53 9	II B	2500
19	53	57 8	II B	1700
20	40	55 0	I B	2800
21	61	91 5	II B	1800
2?	50	55 0	II A	2800
23	50	66 0	II B	3350
24	60	80 5	II A	3100
25	55	54 5	III	3850
26	52	86 0	II B	3300
7	52	63 0	II A	4500
8	65	76 0	I B	3000
29	44	50 8	IV	3500
30	36	7_0	II B	3650
31	48	56 _	I B	2700
32	41	69 8	I B	1300
33	54	59 0	II B	3100
34	49	48 2	II B	3550
35	34	6 0	I B	3300
36	41	51 6	I B	3350

Table V *S OCT (nanomoles) in 20 women before and 1 4 6 and 8 days after start of radiation treatment*

Case	Initial level	Days after start of radiation treatment			
	0	1	4	6	8
17	0 7	0 5	2 8	8 2	1 7
18	0 5	0 6	1 1	0 8	1 0
19	1 1	9 9	2 3	1 5	0 8
20	1 2	0 3	4 2	1 9	0 8
21	3 6	7 5	26 0	6 ?	4 3
2?	0 9	1 5	2.0	1 4	0 7
23	0 6	0 5	2.6	2 5	4 6
24	1 7	1 0	1 3	1 6	2 3
25	1 1	1 6	4 4	1 7	1 9
26	2 1	2 0	2 3	2 3	1 9
27	1 1	1 8	14 7	9 7	6 ?
28	1 8	0 6	11 3	2 5	1 0
29	0 4	1 1	2 7	1 0	0 9
30	1 0	2.6	0 4	0 6	0 8
31	0 6	0 4	1 5	1 7	0 4
32	3 5	7 2	3 3	3 3	4 0
33	2 0	2 0	3 0	12 5	10 9
34	0 9	0 4	4 5	2.4	1 5
35	0 6	0 3	0 3	1 9	0 8
36	0 6	1 5	1 9	0 8	0 9
Mean	1 3	2 _	4 6	3 2	2 4
S D	0 9	2.7	6 1	4 2	6
Range	0 4–3 6	0 3–9 9	0 3 26 0	0 6–1 5	0 4–10 9

Table VI *Urea nitrogen in serum (mg/100 ml) in ?0 women before and 1 4 6 and 8 days after start of radiation treatment*

Cas	Initial level 0	Days after st rt of radiation treatment 1	4	6	8
17	13	19	16	13	17
18	9	11	14	11	14
19	11	15	13	12	13
0	14	0	16	14	14
?1	14	0	13	1?	13
-2	13	1	16	1	21
3	1	19	13	13	14
3	13	--	17	19	15
-5	9	15	9	11	
	11	17	11	10	7
?	13	15	13	11	10
	11	?3	13	10	17
9	15	21	15	17	17
30	8	14	15	10	8
31	11	?1	12	1?	10
3	11	14	13	11	11
33	14	-0	13	10	10
34	13	13	11	11	9
35	10	15	14	1?	13
3r	9	15	10	9	9
Mean	1	18	13	12	13
S D	-0	3 5	2 1	3 1	3 7
Rang	8 15	11 3	9 17	9 ?1	?- 1

The average amount of urea nitrogen in serum was 12 mg 100 ml b fore radiation It had risen to 18 mg 100 ml 24 hours after th- start of radiation this increase is statistically significant ($p < 0.001$) After this there was a gradual return to the initial level (Table VI)

DISCUSSION

Operation trauma (19) as well as radiation of malignant tumours (24) elicits a breakdown of protein and an elevated excretion of nitrogen in the urine It has b--n suggested that it is this breakdown of protein that is responsible for the rise in S-OCT that occurs 4–7 days after an op ra tion trauma (4) This theory is support-d by the present finding that radiation of malignant tu mours also elicits a late rise in S-OCT (on the 4th–6th day) At first sight it seems that the post traumatic increase in adrenocortical activity might also lie behind the late rise in S-OCT after opera tion or radiation If this were th case however the ten subjects who received 90 IU ACTH on three consecutive days should also have displayed a rise in S-OCT since the adreno-ortical stimula tion in this group was presumably approximately the same as that which follows operation trauma—both an operation with moderate destru-tion of tissue (4) and the intravenous administration of 75 IU ACTH during 8 hours (3) have been shown to elicit an approximately threefold increase in the urinary excretion of 17 hydroxycorticosteroids Thus a stimulation of the adrenal cortex does not appear to suffice by itself as an explanation of the rise in S OCT 4–7 days after operation trauma and 4–6 days after radiation of malignant tu mours

Balance studies in the rat thirty years ago showed that the administration of gluco orti o-steroids elicited an increased breakdown of pro-tein and a rise in the urinary excretion of nitrogen (17) In the present study on the other hand the breakdown of protein was not increased by stimu lation of the adrenal cortex (cases 11–16) which is in keeping with other reports (10) The reason for this discrepancy is probably that the increas in the production of glucocorticosteroids elicited by physiological stimulation of the adrenal cortex is too slight to result in an increased breakdown of protein In studies where gluco-orticosteroids were administered to mice the nitrogen balance became negative if the dose exceeded the maximal endogenous production but did not change if the dose was more moderate (15) There is also a qualitative difference between the pharmacologi cal administration of glucocorticosteroids and physiological stimulation of the adrenal cortex in that th- latter also increases the production of steroids with a potentially anabolic effe t (e g 17 alphahydroxyprogesteron) and this is con sidered to promote the build up of protein (10) Thus a stimulation of the adrenal cortex do-s not by itself appear to explain why the breakdown of protein and the excretion of nitrogen in rease af ter an operation trauma or the radiation of ma lignant tumours This circumstance has also b-en pointed out before (20)

Th- numerically slight but statisti ally sig nificant drop in serum urea after adreno-ortical stimulation (cases 1–10) could b- due to th- retention of sodium and water as a result of the stimulation In this context however on- must bear in mind that th se subjects did not eat a standardised diet with a constant nitrog n content and consequ-ntly the administration of ACTH may have affected the subjects choi-e of food It

would have been preferable of course if the study of nitrogen balance had been made in the subjects (cases 1–10) from whom blood was sampled This was not feasible however partly because most of these subjects were unwilling to participate in such a study

It is still not entirely clear what elicits the drop in S-OCT after stimulation of the adrenal cortex (cases 1–10) One possible cause may be that corticosteroids have been shown to stabilise certain cellular and sub cellular membranes (13 26) and the resultant reduction of permeability may conceivably lead to a diminished release of intracellular enzymes (including OCT) into the blood stream

Several explanations may be put forward for the rise in S-OCT 4–6 days after radiation therapy (cases 17–36)

A In view of the time course it seems somewhat unlikely that the radiation has a direct effect on the liver with the release of OCT as a result For one thing increases in serum transaminase elicited in this way in the rat were found to occur only a few hours after the radiation (12) Even a latent radiation effect seems somewhat improbable in view of the rapid decline of the radiation dose from the intracavitary irradiators (14) and the relatively large distance between the liver and the radium deposit

B A liver hypoxia during anaesthesia also appears to be a somewhat unlikely explanation in view of the time course—liver hypoxia in connection with an operation has been found to manifest itself in a rise of S OCT after 24 hours (6) Furthermore none of the women displayed any clinical signs of circulatory failure during the therapy

C Hepatotoxic breakdown products can be formed in necrotic tissue (8) which might explain the rise in S-OCT If this were the sole cause however a greater number of the women should have displayed a rise in S OCT after 24 hours since the decomposition of tissue starts relatively soon after irradiation (1) Hepatotoxic substances however may have contributed to the high level of S-OCT on days 4 and 6 at least in the three women (cases 19 21 and 32) in whom a rise of S-OCT was observed after only 24 hours Even without these three subjects however the rise in S OCT on day 4 and day 6 is statistically significant

D Another possible explanation of the rise in S OCT on days 4–6 is that the increased breakdown of nitrogen by adding to the load on the liver cells elicited a greater cellular permeability for e g OCT If so however S-OCT should have been higher on day 1 than on days 4 and 6 since judging from the level of serum urea the breakdown of nitrogen was more marked on day 1 Unfortunately it was not possible to study the nitrogen balance in these women (cases 17–36) because they were hospitalised for too short a time

E It seems probable that the increased breakdown of protein induced a greater synthesis of OCT in the liver as a result of a heavier load on the enzymes in the urea cycle Strong support for this theory is to be found in animal experiments by Schimke (22 23) showing that states involving an increased breakdown of protein in the rat result in a greater amount of OCT in the liver After a few days when the breakdown of protein diminished OCT was no longer required to the same extent for which reason some of the surplus enzyme may have left the liver cells Support for this theory may be derived from the observation that a marked increment of protein to the human diet results in a rise in S-OCT that reaches a maximum during the first few days after this increment has been withdrawn (5) Studies of the nitrogen balance have shown that the breakdown of protein is elevated after local radiation therapy for cancer of the uterine cervix the breakdown of tissue was found to correspond to approximately 2 g nitrogen per 24 hours during the first few days after the radiation (24)

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CIRCULATING ANTIBODIES IN GASTRITIS

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Abst act The presence of serum parietal cell and intrinsic factor antibodies has been examined in 51 subjects including eight patients with pernicious anemia Parietal cell antibodies were found only in patients with atrophic gastritis (in 16 out of 30) and mainly in thos with complete or almost complete loss of parietal cells (in 14 out of 20) Blocking intrinsic factor antibodies were present only in the latter group (in 8 out of 20) Binding intrinsic factor antibodies were found only in one patient with complete loss of parietal cells Parietal cell and intrinsic factor antibodies were found in patients with pernicious anemia (in respectively 6 and 4 out of 8) as well as in patients with s vere atrophic gastritis without pernicious anemia (in respectively 8 and 4 out of 12) There is a good correlation between the results of the Schilling test and the occurrenc of these antibodies. No correlation is observed between the severity of lymphocyte and plasma cell infiltration and the occurrence of parietal cell antibodies, whereas a reverse correlation exists with *the occurrence and the titer of intrinsic factor antibodies*

A long term follow up of subjects with various conditions of the gastric mucosa suggests that atrophic gastritis might be one of the factors which predispose to gastric carcinoma and pernicious anemia (12) Atrophic gastritis is how ever a very common condition its prevalence rate in a randomly selected Finnish rural population is 28 % (13) Hence only a few subjects with atrophic gastritis have a possibility to develop gastric carcinoma or pernicious anemia The question th refore arises why some of the patients with atrophic gastritis develop gastric carcinoma others pernicious anemia whereas still others and these probably are the majority suffer only from simple atrophic gastritis Consequently a closer characterization of atrophic gastritis is necessary

The occurrence of parietal cell and intrinsic factor antibodies has been demonstrated in atrophic gastritis with or without pernicious anemia (1 5 6 8 10 14 15 16) We have examined sera of subjects with various types and degrees of gastritis for the occurrence of antibodies to intrinsic factor and parietal cells

The present study forms part of a larger project for investigating the occurrence of parietal cell and intrinsic factor antibodies in the inhabitants of a Finnish rural commune and in addition in subjects selected for genetic studies

MATERIAL AND METHODS

Parietal cell and intrinsic factor antibodies were examined in 51 patients Of these 17 were outpatients, who were selected for the present study because they were known to have atrophic gastritis verified by biopsy The remaining 34 patients, including eight with pern cious anemia, were selected from patients treated during 1966–1967 at the Second Department of Medicine for various gastroenterologic diseases.

Gastric biopsies were performed with a Sielaff suction biopsy tube (Richard Wolf West Germany) In all 190 specimens were obtained from the body of the stomach of 51 patients. The specimens were fixed in 10% formalin and stained with hematoxylin-eosin

The definitions of variou types and degrees of gastritis are given in Table I and are based mainly on the presence of normal body gland and on the presence of inflammatory cells.

Blocking antibodies to int insic factor were demonstrated and titrated by the charcoal method of Ardeman–Chanarin ( ) *B nd ng antibod es to ntr ns c facto – v tam n B_{12} complex* were examined by gel diffus on combined with autoradiography (6 7) In both methods a vitamin B_{12} labeled with Co^{57} with a *specific activity of 1 mC μg* (Amersham The Rad ochemical Centre England) was used.

Pa ietal cell ant bodies were demonstrated by the indirect immuno-fluorescen e method (3) using bivalent rabbit antihuman IgG IgM globulin conjugated with fluorescein isothiocyanate Cryostate sect ons from a normal

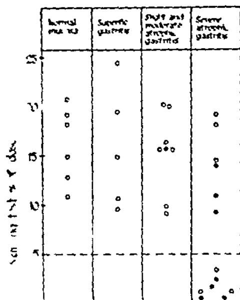


Fig. 1. Relation of parietal cell antibodies to gastritis and absorption of radiovitamin B_{12}. O pcs antibodies absent. ● pcs antibodies present.

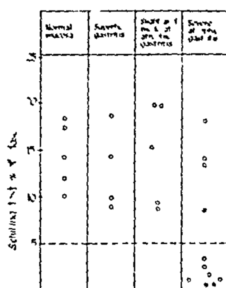


Fig. 2. Relation of intrinsic factor antibodies to gastritis and absorption of radiovitamin B_{12}. ● i.f. antibodies and pernicious anemia. ⊖ i.f. antibodies without pernicious anemia. O no i.f. antibodies.

human body mucosa obtained at gastric surgery were used as antigens. The control reactions consisted either of blocking the cryostate sections treated with the patient's serum by unconjugated rabbit antihuman IgG IgM globulin or of the absorption of the patient's serum with a homogenate of human gastric mucosa (100 mg of dried tissue powder/ml serum). In addition, normal human serum and a positive control serum known to have a high titer of parietal cell antibodies were always included in each test series. The titer of the parietal cell antibodies was determined by using five fold dilutions of patient sera.

Vitamin B_{12} absorption was studied in 40 patients by the ordinary Schilling test (9) using 1 µg of Co labeled vitamin B_{12}. *In case of pathologic findings the test was* repeated.

RESULTS

The occurrence of parietal cell and intrinsic factor antibodies in various conditions of the gastric mucosa is illustrated in Table I. Moreover, the occurrence of these antibodies in relation to the state of the gastric mucosa and to the results of the Schilling test are shown in Figs. 1 and 2. It appears that none of the subjects with superficial gastritis and with normal gastric mucosa had these antibodies. In subjects with slight or moderate atrophic gastritis no intrinsic factor antibodies were found; two of them, however, had parietal cell antibodies. Of the 20 subjects with severe

Table I. *Parietal cell and intrinsic factor antibodies in gastritis*

Histology of the gastric mucosa	No. of cases	Parietal cell antibodies	Intrinsic factor antibodies: Blocking	Binding
Normal mucosa	11	–	–	–
Superficial gastritis[a]	10	–	–	–
Slight or moderate atrophic gastritis[b]	10	2	–	–
Severe atrophic gastritis without pernicious anemia	12	8	4	–
Severe atrophic gastritis with pernicious anemia	8	6	4	1
No. of cases	51	16	8	1

[a] Increase of "inflammatory" cells beneath the surface or throughout the mucosa with normal amounts of tubules containing parietal cells.
[b] Slight or moderate loss of parietal cells containing tubules.
[c] Complete or almost complete loss of parietal cells.

atrophic gastritis (complete or almost complete loss of parietal cells) 14 had parietal cell antibodies and eight blocking and one binding intrinsic factor antibodies Hence these antibodies were found only in atrophic gastritis and mainly in its most severe form Further Table I shows that both antibodies were observed in severe atrophic gastritis both with and without pernicious anemia parietal c ll antibodies were found in these groups in respectively 75 and 67 and intrinsic factor antibodies in respectively 50 and 33 %

There was a close correlation between the occurrence of these antibodies and the results of the Schilling test as shown in Figs 1 and 2 Of the 26 subjects with normal Schilling test five had parietal and one blocking intrinsic factor antibodies Of 14 subjects with low absorption of radiovitamin B_{12} 11 had parietal cell antibodies and s ven blocking and one binding intrinsic factor antibodies

No correlation was observed between the occurrence and titers of parietal cell antibodi s and the severity of plasma cell and lymphocyte infiltration and the presence of germinative center reaction On the other hand both the occurrence and titers of blocking intrinsic factor antibodies showed a reverse correlation to the severity of inflammatory signs (Fig 3) Thus in the atrophic group intrinsic factor antibodies were found in six out of ten cases with slight inflammatory infiltration and in only two out of 20 with moderate or extensive inflammatory infiltration Of the ten subjects with atrophic gastritis associated with slight inflammatory signs nine had complete or almost complete loss of parietal cells and could accordingly be classified as gastric atrophy There was also a tendency for inflammatory infiltration to decrease with increasing loss of parietal cells

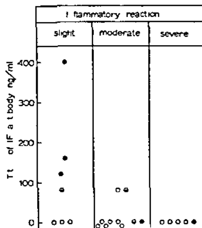


Fig 3 Relation of intrinsic factor antibody titers to the inflammatory reaction in the gastric mucosa and to the results of Schilling test in patients with atrophic gastritis O Schill ng test above 5 of the dose given ⊖ S h lling test below 5 % of the dose without pernicious anemia ● pernicious anemia.

DISCUSSION

The occurrence in the present series of parietal cell antibodies only in subjects with gastritis suggests some causal relation between these phenomena During destruction of parietal cells some change may occur in the structure of their proteins in such a way that they are regarded as antigens by immunocompetent cells of the body with consequent antibody formation This assumption seems to be refuted however by the fact that the present method enabled the antibodies to react with *normal* parietal cells On the other hand it is possible that because of the localization structure and function of parietal cells no tolerance to them develop d during foetal life Hence a destruction of parietal cells causing a release of their proteins into the neighbouring area or the blood may lead to antibody formation

Parietal cell antibodies may also be of some etiologic significance since they have been observed by other authors in some subjects with a normal stomach or superficial gastritis (16) In our study no parietal cell antibodies were found in these conditions of the gastric mucosa but since they were only 21 no conclusions can be drawn regarding the etio ogic significance of these antibodies on the basis of the present study A long term follow up of subjects with normal mucosa associated parietal cell antibodies might solve the problem

The presence of intrinsic factor antibodies only in patients with complete or almost complete loss of parietal cells suggests that they are the result rather than the cause of the process which leads to intrinsic factor deficiency Intrinsic factor is presumably produced by parietal cells (4) s c reted directly into the gastric lumen and not ab

sorbed by the intestine This may explain its "inaccessibility" to immunocompetent cells of the body Hence its release into the blood as a result of destruction of parietal cells may lead to antibody formation Another alternative would be production of an intrinsic factor which differs sufficiently from normal to induce antibody formation

There was no correlation between the presence of parietal cell antibodies and the degree of "inflammatory" infiltration in the gastric mucosa On the other hand intrinsic factor antibodies showed a reverse correlation with the severity of "inflammatory" signs these antibodies occurred mainly in subjects with atrophic gastritis who showed only slight or almost total absence of lymphocyte and plasma cell reaction This finding conflicts with the results obtained by Schwartz (11) who found intrinsic factor antibodies only in those pernicious anemia patients with marked "inflammatory" changes It should also be noted that the "inflammatory" changes tended to diminish with increasing loss of parietal cells

From our study it appears that severe atrophic gastritis defined as complete or almost complete loss of parietal cells is characterized by 1 absence of severe plasma cell and lymphocyte infiltration 2 low absorption of radiovitamin B_{12} and 3 presence of parietal cell and intrinsic factor antibodies

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ATRIAL FIBRILLATION

I A Study of Atrial Thrombosis and Systemic Embolism in a Necropsy Material

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Abstract The incidence of embolism and atrial thrombosis in a necropsy material of patients with atrial fibrillation was studied In all 642 patients were reviewed. The incidence of embolism was high and significantly higher ($p < 0.05$) in the group with valvular heart disease and congenital heart lesions compared to a group with mainly arteriosclerotic and hypertensive heart disease

The occurrence of embolism was not correlated to the number of conversions to sinus rhythm in either group On the other hand there was a lower incidence of embolism in atrial fibrillation of short duration compared to long duration This difference however was significant ($p < 0.001$) only in the non valvular group

These results are briefly discussed with regard to the indications for conversion of atrial fibrillation

The direct current countershock technique to convert atrial fibrillation (AF) has many advantages compared to quinidine The convertibility rate is up to twice as high and this method is not as time-consuming (13 15 18 19) The consequence is that more patients will be treated and each patient will be treated several times

Of the different complications to AF the most serious one is that of systemic embolism As there is a diversity of opinion concerning the risk of embolization accompanying the conversion procedure compared to that of AF per se the importance of more knowledge on this question has increased with the new technique (1 2 8 17 18)

The purpose of this investigation was to study a necropsy material of cases with AF with regard to the frequency of atrial thrombosis and embolism in general More particularly the aim was to study the frequency of embolic episodes in relation to the number of relapses as well as to the duration of the arrhythmia

MATERIAL AND METHODS

The study was performed at the University Hospitals of Umeå and Uppsala. All records of patients who died in the Departments of Medicine during 1955–1964 were reviewed The total number was 3767 of whom 14 9 from Umeå and 2338 from Uppsala The next step was to select those patients who had had AF at any time during the final and/or earlier admissions to the hospitals Thus the material was limited to 693 patients, of whom 246 from Umeå and 447 from Uppsala The clinical records of seven patients with AF were missing and these patients were excluded. The diagnosis of AF in these cases was known after checking in the files of electrocardiographic (ECG) tracings at the Department of Clinical Physiology Forty four patients were also excluded as no autopsy or an incomplete one was performed Thus the final material consisted of 64_ patients. There were 33_ males and 310 females The mean age at death was 69 0 years range 19–97 years.

The clinical records of these patients were carefully reviewed as to the number of conversions and the duration of the arrhythmia

A conversion, whether obtained spontaneously by drugs or countershock, was accepted only when documented on ECG The conversions registered are consequently a minimum number

The duration was recorded as the time from which the patient first experienced a definitely irregular heart activity Without such a definite history the time since the physician first diagnosed AF was used. In the vast majority of patients it was necessary to use the time for the first ECG revealing AF In case of recurrent episodes of AF the total duration was recorded

The autopsy reports were studied with regard to heart disease the occurrence and localization of atrial thrombosis and/or arterial embolism.

In view of the difficult pathologic anatomic diagnosis of old cystic cerebral malacias with regard to the origin the diagnosis of cerebral embolism has been applied to all of them, without or with minimal hemorrhage (1) The diagnosis of embolies at other sites is easier In arteries to the extremities the embolies have not been diagnosed without a clinical history compatible with an embolic episode The reason is an attempt to differentiate

Table I *The different valvular lesions in the group with rheumatic heart disease*

		No of pats.	
		Total	Operated
Mitral	stenosis	16	1
	regurgitation	4	
	combined	22	2
Aortic	stenosis	4	
	regurgitation	3	
	combined	8	
Mitral aortic lesions		38	3
Three valvular lesions		9	
Grand total		95	6

between embolus and thrombosis on or adjacent to a sclerotic plaque

Concerning the anticoagulation therapy during the AF there was no uniform principle during the time for this retrospective study except for acute myocardial infarction where dicumarol has been given. In the group with valvular disease or congenital heart lesions (VOC) less than 10 of the patients have had anticoagulation therapy more than two weeks.

The different etiol gi groups were limited to three The VOC group mainly consisted of rheumatic valvular disease of mostly severe degree There were also one patient with luetic heart disease one patient with severe aortic stenosis due to atheromatosis and four patients with congenital heart lesions. One patient had ventricular septal defect one atrial septal defect one bicuspid aortic valve with regurgitation, and finally one had patent ductus arteriosus with endocarditis. The different valvular lesions in the patients with rheumatic heart disease diagnosed clinically as well as at the necropsy are shown in Table I The mean age in the VOC group was 60.4 years.

Table II *The composition of the group "others" with reference to established or unknown cardiac disease*

	No of pats.
Myocardial infarction	04
Moderate to severe coronary sclerosis without myocardial infarction	100
Hypertensive cardiovascular disease	
With myocardial infarction	31
Without myocardial infarction	54
Malignancies with little or no coronary sclerosis	18
Cor pulmonale	4
Miscellaneous (ASHD[a] infections, uremias etc)	95
Total	506

[a] The degree of coronary sclerosis was mild.

To the second group VOC+ASHD (arteriosclerotic heart disease) belonged only cases with rheumatic valvular disease and recent or old myocardial infarction. The mean age in this group was 67.0 years.

All remaining cases were included in the group "others" (later in text referred to as the third group) in which the dominating heart diseases were coronary artery sclerosis and or hypertensive cardiovascular disease The composition of this group is shown in Table II The mean age was 70.9 years.

Every patient had a control subject of the same sex, same age ± 2 years and the same year of autopsy but without AF They were chosen at random from the files of autopsy reports in the Institutions of Pathology in Umeå and Uppsala

The ideal situation, to have a control material with the same diagnosis as well as similar duration of the disease was unobtainable particularly for the VOC patients. The reason was the lack of patients with VOC in the autopsy materials from the hospitals reported and the natural course of some types of VOC to develop AF after a certain interval. Consequently the same ratio of diagnoses within the control group and the material studied was not possible to obtain The number of patients with arteriosclerotic and hypertensive heart disease was about the same in the control material and the AF material. However there was a decreased number of patients with VOC in the control group Twenty-eight patients with VOC were included which is less than 30 of that in the AF material Correspondingly patients with malignancies were more numerous in the control material.

STATISTICAL METHODS

The following probability levels have been used $p < 0.001$ highly significant $0.001 < p < 0.01$ significant and $0.01 < p < 0.05$ probably significant The formulas for calculation of the significance between numbers and proportions are those described in current textbooks on statistics (11)

RESULTS

Atrial thrombosis

In the entire material the occurrence of atrial thrombosis is shown in Table III It is likely that these results represent minimum numbers Some atrial thrombi might be overlooked particularly if the atrial thrombus had disappeared with the embolus only leaving microscopic changes in the atrial endocardium The difference between the number of atrial thrombi in the VOC group and the third group is significant * The more frequent occurrence of atrial thrombi in the left atrium compared with the right is obvious The levels of significance are given in the table

The number of atrial thrombi without embolism is surprisingly constant in all groups

Table III *Occurrence of atrial thrombosis in the different etiologic groups and in the control material*

Abbreviations L A left atrium and R A right atrium Numbers within brackets are percentages

	No of pats	Atrial thrombosis L. A only	R A only	LA/RA significance	L A + R A only	Total	Without emboli
1 VOC	101	21 (0 8)	4 (–)		9 (8 9)	34 (33 7)	8 (23 5)
VOC + ASHD	35	6 (–)	3 (–)	—	2 (–)	11 (31 4)	3 (–)
3 Others	506	51 (10 1)	25 (4 9)		13 (2.6)	89 (17 6)	23 (25 8)
Grand total	64?	78 (12 1)	32 (5 0)		4 (3 7)	134 (0 8)	34 (25 4)
Control	642	8 (1 2)	4 (–)	—	3 (–)	15 (3)	5 (–)

In groups 2 and 3 there are one and five patients respectively with atrial thrombosis combined with ventricular mural thrombosis

Table IV *Location of the thrombus within the atrium*

Numbers within brackets are percentages

		1 VOC	2 VOC – ASHD	3 Others	Total	Controls
No of pats.		101	35	506	642	642
Atrial thrombosis		34 (33 7)	11 (31 4)	89 (17 6)	134 (20 8)	15 (2 3)
Location						
Only left atrium	main wall	9 (26 5)	2 (–)	12 (13 5)	23 (17 2)	1 (–)
	appendage	10 (29 4)	2 (–)	35 (39 3)	47 (35 1)	7 (46 7)
Only right atrium	main wall	2 (–)	1 (–)	4 (–)	7 (5 2)	1 (–)
	appendage	2 (–)	1 (–)	18 (0 2)	21 (15 7)	3 (–)
Both appendages		5 (14 7)	1 (–)	8 (9 0)	14 (10 4)	3 (–)
Both atria		2 (–)	1 (–)	4 (–)	7 (5 2)	
Other combinations		4 (–)	3 (–)	8 (9 0)	15 (11 2)	

(Location rows bracketed: 100)

The site of the atrial thrombosis within the atria is tabularized separately in Table IV

The incidence of embolism correlated to the site of the atrial thrombosis in the left atrium is shown in Table V There is no apparent trend but the small groups should be rem mbered

Embolism

The occurrence of embolism at various sites is shown in Table VI The different groups of em bolism alone or combin d are listed according to the degree of reliability of the pathologic anatomic diagnosis (12)

The pulmonary artery emboli are not included as they more frequently originate from venous thrombi in the legs than from the heart even in cardiac patients Table VII shows the occurrence of pulmonary emboli

In Table VIII a comparison is made b tween the embolism in the VOC group of the AF material and VOC patients of the control material The difference is significant

In the third group there were 41 patients with malignant disease of whom a noticeable number were of fairly young age Eighte n patients de

Table V *Incidence of embolism correlated to the site of left atrial thrombosis*

	Site of atrial thrombosis (l ft atrium)	No of pats with atrial thrombosis	with systemic embolism
1 VOC	Main wall	9	5
	Appendage	10	8
2. VOC ASHD	Ma n wall	2	2
	Appendag	2	1
3 Others	M n wall	11	9
	Appendage	31	0
Total	Main wall	–	16
	Append ge	43	9

Table VI *Occurrence of embolism at various sites in the different etiologic groups and in the control material*
Numbers with n bra kets are percentages

	Diagnosis				
	1 VOC	? VOC + ASHD	3 Others	Total	Controls
No of pats.	101	35	506	64	64
Type of embolism					
(a) Splenic renal peripheral or abdominal artery cerebral or 3 of them	13	1	30	44	4
(b) Splenic renal	1	1	3	5	4
(c) Splenic cerebral	1	2	11	14	3
(d) Renal cerebral	8	4	23	35	
(e) Peripheral or abdominal artery + other embolus (not pulmonary)	4	3	11	18	7
(f) Peripheral and or abdominal artery	-		6	8	5
(g) Splenic	2	2	10	14	4
(h) Renal	12	2	18	3	27
(i) Cerebral	11	4	99	114	66
Grand total	54 (53 5)	19 (54 3)	11 (41 7)	784 (44 2)	1 0 (18 7)

veloped AF at the final stage of their disease without presenting any cardiac disease at the autopsy

Recurrent conversions

In Table IX a comparison is made b tween patients having recurrent conversions and those having permanent AF The distribution of conversions within the entire material is seen in Fig 1

There was no significant difference in any of the groups comparing patients with recurrent conversions and those with permanent AF

Duration of AF

There is a definite relation between the duration of the arrhythmia and the incidence of embolism only in the third group as seen in Table X The difference between the percentage of embolism in AF of short duration to that of moderately long and long duration is highly significant * This result is also valid for the total material

DISCUSSION

This investigation was performed at two different hospitals The reason was the desire to obtain as

Table VII *Occurrence of pulmonary embolism*

	Diagnosis				
	1 VOC	2 VOC ASHD	3 Others	Total	Controls
No of pats.	101	35	506	64	64
Pulmonary embolism combined with other embolism					
Pulmonary embolus with venous thrombosis	6		30	36	1
Pulmonary embolus without venous thrombosis	6	1	6	33	9
Pulmonary embolism alone					
Pulmonary embolus with venous thrombosis	3	1	11	15	46
Pulmonary embolus without venous thrombosis	8	3	13	4	3
Grand total	23	5	80	108	1C

Table VIII *Embolism in the VOC groups*

Numbers within brackets are percentages

	No of pats	Systemic
VOC patients in the material studied	101	54 (53 5)
VOC patients in the control material	28[a]	10 (35 7)

Patients with VOC and myocardial infarctions are not included

large a material as possible in order to get sub groups of significant size for the purpose of this study I do not feel although the hospital popula tions are different in Umeå and Uppsala that it is inaccurate to combine these in view of the special aim of this investigation In an epidemiologic and clinical study this would certainly not be possible in the same way

Therefore it is interesting to find almost the same frequency of AF in the total number of deaths in the two hospitals The percentage in Umeå was 17 2 and in Uppsala 19 1

The pathogenesis of atrial thrombosis is not completely understood Several factors have been implicated AF may predispose hemodynamically being a stagnation factor of the blood from the atria (4 22) Localized atrial endocarditis is con

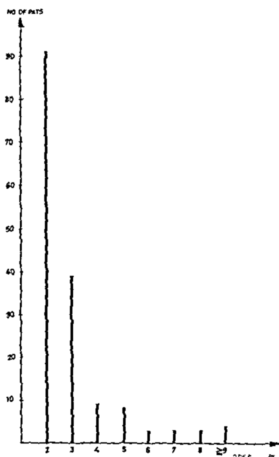


Fig 1 Distribution of minimum number of conversions

Table IX *Embolism correlated to the number of known conversions*

A permanent atrial fibrillation or one conversion *B* more than one conversion

Etiology	No of pats	Embolism Cerebral	Other type and multiple	Total No of pats.	
1 VOC					
A	83	8	43	51	61 4
B	18	1	13	14	77 8
2 VOC - ASHD					
A	30	4	16	20	66 7
B	5		3	3	60 0
3 Others					
A	374	51	128	179	47 9
B	132	17	39	56	42.4
Total					
A	487	63	187	250	51 3
B	155	18	55	73	47 1

Table X *Embolism related to the duration of atrial fibrillation*

The total number is reduced by those with unknown duration of the arrhythmia

Etiology and duration of atrial fibrillation	No of pats	Embolism No of pats.	
1 VOC			
< 1 week	9	5	55 6
1 week–3 years	41	24	58 5
> 3 years	38	25	65 8
2. VOC + ASHD			
< 1 week			
1 week 3 years	16	12	75 0
> 3 years	15	8	53 3
3 Others			
< 1 week	126	47	37 3
1 week–3 years	.39	129	54 0
> 3 years	62	34	54 8
Total			
< 1 week	135	52	38 5
1 week–3 years	296	165	55 7
> 3 years	115	67	58 3

Table VI *Occurrence of embolism at various sites in the different etiologic groups and in the control material*
Numbers within brackets are percentages

	Diagnosis				
	1 VOC	2 VOC+ASHD	3 Others	Total	Controls
No of pats	101	35	506	642	642
Type of embolism					
(*a*) Splenic + renal + peripheral or abdominal artery + cerebral or 3 of them	13	1	30	44	4
(*b*) Splenic + renal	1	1	3	5	4
(*c*) Splenic + cerebral	1	2	11	14	3
(*d*) Renal + cerebral	8	4	23	35	
(*e*) Peripheral or abdominal artery + other embolus (not pulmonary)	4	3	11	18	7
(*f*) Peripheral and/or abdominal artery	2		6	8	5
(*g*) Splenic	2	2	10	14	4
(*h*) Renal	12	2	18	32	27
(*i*) Cerebral	11	4	99	114	66
Grand total	54 (53 5)	19 (54 3)	211 (41 7)	284 (44 2)	1 0 (18 7)

veloped AF at the final stage of their disease without presenting any cardiac disease at the autopsy

Recurrent conversions

In Table IX a comparison is made between patients having recurrent conversions and those having permanent AF The distribution of conversions within the entire material is seen in Fig 1

There was no significant difference in any of the groups comparing patients with recurrent conversions and those with permanent AF

Duration of AF

There is a definite relation between the duration of the arrhythmia and the incidence of embolism only in the third group as seen in Table X The difference between the percentage of embolism in AF of short duration to that of moderately long and long duration is highly significant*** This result is also valid for the total material

DISCUSSION

This investigation was performed at two different hospitals The reason was the desire to obtain as

Table VII *Occurrence of pulmonary embolism*

	Diagnosis				
	1 VOC	2 VOC+ASHD	3 Others	Total	Controls
No of pats	101	35	506	642	642
Pulmonary embolism combined with other embolism					
Pulmonary embolus with venous thrombosis	6		30	36	17
Pulmonary embolus without venous thrombosis	6	1	26	33	9
Pulmonary embolism alone					
Pulmonary embolus with venous thrombosis	3	1	11	15	56
Pulmonary embolus without venous thrombosis	8	3	13	24	37
Grand total	23	5	80	108	109

the group of AF of short duration This circum stance lends forceful support for early conversion at least in cases with atrial fibrillation due to arteriosclerotic and/or hypertensive heart disease

The immediate treatment of AF occurring during an acute myocardial infarction recommended by Lown and others is important not only from hemodynamic points of view but also to prevent embolism (16)

Long term anticoagulation prophylaxis has not been given routinely to any group except to patients with concomitant myocardial infarction In rheumatic heart disease with AF there is already agreement as to the indication for anticoagulation therapy (21) In cases with AF but no rheumatic heart disease most authorities have not earlier given anticoagulation therapy (2) The high incidence of embolism in my material in this group is difficult to accept There is obviously reason to reevaluate the anticoagulation therapy for AF of non valvular cause

The suspicion that has arisen in the literature that recurrent conversions of atrial fibrillation might influence the incidence of embolism (2 7 8 18 20) has not been confirmed in this study As the conversions have mainly occurred spontaneously or by drugs my conclusion should be limited to those types of conversion Only very few had countershock within the material However I would believe that the same conclusion can be drawn for conversions performed by countershock technique

ACKNOWLEDGEMENTS

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ATRIAL FIBRILLATION

II *A Study of Fibrillatory Wave Size on the Regular Scalar Electrocardiogram*

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Abstract The amplitude of the *f* waves has previously been correlated to the etiology of the atrial fibrillation and to the size of the left atrium. This investigation has revealed coarse *f* waves to occur more frequently than fine *f* waves in rheumatic heart disease which is in agreement with earlier reports In arteriosclerotic heart disease however fine *f* waves occurred in only 21% which is in opposition to the result of most earlier reports

No correlation between the amplitude of the *f* waves and the left atrial size was found

It is concluded that in the individual case no safe conclusions can be drawn from the size of the *f* waves in conventional ECG leads

The size of the fibrillatory waves (*f* waves) has previously been correlated to different etiologies of atrial fibrillation (AF) Coarse *f* waves are described as occurring in rheumatic and congenital heart disease whereas fine *f* waves are reported in arteriosclerotic heart disease (2 4–6 9 10 13–16)

Some authors have suggested that coarse *f* waves may be explained on the basis of distention of the atria (15 16) In accordance with this concept the size of the *f* waves particularly in lead V_1 or CR_1 may be used as an electrocardiographic sign of atrial enlargement and in most cases left atrial enlargement.

This investigation was undertaken to study the *f* wave size with regard to etiologic diagnosis in cases belonging to a necropsy material which will be reported on concerning atrial thrombosis and systemic embolism (1)

Another purpose was to study the *f* wave size in relation to the pressure and the size of the left atrium in a clinical material

MATERIAL AND METHODS

The study was performed at the University Hospitals of Umeå and Uppsala All records of patients who died in the Departments of Medicine during 1955–1964 were reviewed. Only patients who had had AF at any time were studied further The number was 642 patients. All patients with digitalis or other heart medication prior to the first ECG with AF were excluded Two-hundred and twenty patients then remained for the *f* wave study Fifty-one of these patients were followed after having been digitalized in an attempt to evaluate the effect of digitalis on the *f* waves The reduced number of patients in this group is mainly due to the requirement that only patients with a minimum of three ECGs after digitalization, of which at least two after completed digitalization, have been included

The amplitude of the *f* waves on an ECG tracing is not uniform but a certain pattern of wave form can be made out, particularly in V or CR_1, where the waves have their greatest amplitude The *f* wave has been measured from the trough to its peak. The measurement has exclusively been performed outside the ST-U regions to avoid influence by the T and U waves The center of the inscription line has been the exact point to start the measurement. A test of 1 millivolt giving a 10 mm deflection has been required.

According to earlier reports, *f* waves are considered *coarse* when exceeding 0.5 mm and *fine* when 0.5 mm or less (16) They are graded *inconsistent* if they changed from coarse to fine or vice versa on different ECGs within the entire series of ECGs for that patient. Fig 1 shows the different *f* waves as well as the calculation procedure

The *f* waves have been measured in patients with different etiology of AF The cases have been grouped according to the diagnostic criteria listed below

Rheumatic heart disease (*RHD*) has been diagnosed when the autopsy revealed rheumatic endocarditis with significant valvular lesions

Arteriosclerotic heart disease (*ASHD*) has been diagnosed in patients with pathologic anatomic evidence of one or more myocardial infarctions (8)

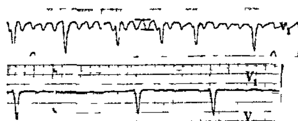


Fig 1 The ECG tracing above shows coarse *f* waves and the calculation of the *f* wave size while the tracing below shows fine *f* waves

Hypertensive cardiovascular disease (HCVD) has been diagnosed in patients with a well documented clinical history of hypertension and autopsy findings compatible with the diagnosis Some cases have had both hyp r tension and myocardial infarction and these are tabular ized separately

Finally there is a group *others* with AF due to other causes than those above The composition in part is shown in another article (1)

To study the *f* waves in rheumatic valvular disease in relation to pressure and size of the left atrium a clinical material was added All patients with AF who had had left heart catheterization (transseptal technique) and left atrial angiocardiography in Uppsala from 1964 and to April 15 1968 were included in this material The left atrial mean pressure at rest was obtained from the catheterization report The size of the left atrium was measured according to Arvidsson s formula (3) The cal culation was performed with the left ventricle in systole

The ECGs were recorded with different types of direct writing machines from Elema Schonander (Elema Scho nander Ltd Stockholm) such as Junior Triplex and Mingograph 42 The vast majority of ECGs were taken with Mingograph 42 (linearity ± 10 mm and flat frequency response up to 500 c/s) The paper speed used was 50 mm/sec

Table I *The f wave size in the different etiologic groups*

Abbreviations RHD = rheumatic heart disease ASHD = arteriosclerotic heart disease HCVD = hypertensive cardio vascular disease

Etiology	No of pats	*F* wave size (mm) 0–0 5	0 5–1	1–2 5	2 5	Incon sistent
RHD	30	8	7	8	4	3
ASHD	56	12	23	15	1	5
HCVD						
With myo cardial infarction	11	2	5	2	1	1
Without myocardial infarction	16	5	7	2	1	1
Others	107	32	34	27	5	9
Total	220	59	76	54	12	19

RESULTS

The *f* wave size within the different etiologic groups is shown in Tables I and II and in Fig 2 The reason for using more than two groups of *f* waves is that some authors use different limits for fine *f* waves (10)

In the autopsy group with RHD coarse *f* waves occurred in 63 % whereas the patients with ASHD had coarse *f* waves in 70% The corresponding numbers in the remaining groups HCVD and others were 67 and 62 % respectively The differences between these percentages are not sig nificant (Statistical methods as in ref 1)

With regard to mean age for patients with dif ferent *f* waves there is a trend to higher mean age in patients with fine compared to patients with coarse *f* waves The differences in mean age are not significant however

In the clinical material with left heart catheter ization (Table III) coarse *f* waves occurred in 89 % The mean age in this clinical material was

Table II *The f wave size correlated to mean age*

Abbreviations as in Table I

	RHD	ASHD	HCVD	Others
Entire material				
No of pats	30	56	27	107
Mean age at time of 1st ECG with atrial fibrillation (y)	55 9[a]	70 1[b]	66 7[c]	68 4[d]
Mean age at death (y)	60 9	7 4	69 8	71 6
Pats with fine f waves				
No of pats	8	12	7	32
Mean age at time of 1st ECG with atrial fibrillation (y)	56 1	7 5	68 0	66 2
Mean age at death (y)	62 4	76 1	72 4	71 0
Pats with coarse f waves				
No of pats	19	39	18	66
Mean age at time of 1st ECG with atrial fibrillation (y)	55 8	69 1	66 5	69 7
Mean age at death (y)	60 3	70 9	68 6	72.0
Pats with inconsistent f waves				
No of pats	3	5	2	9

[b] [d] four nine five and twenty patients respectively with unknown duration of the atrial fibrillation

51 1 years which should be compared with the mean age for patients with RHD in the total necropsy material namely 60 9 years at death and 55 9 years at the time for the first ECG with AF

As seen in Table III there is no correlation whatsoever between left atrial size or left atrial pressure and *f* wave amplitude

Table IV shows the change of the *f* waves af ter digitalization In less than 8 ′ of the patients did the amplitude of the *f* waves change after digitalization

DISCUSSION

Hewlett and Wilson reported in 1915 a patient having AF with large *f* waves (7) but attached no significance to their finding Cookson was first to discuss the size of the *f* waves in view of different etiologies of AF (4)

In their excellent work on The auricular ar rhythmias Prinzmetal et al described movements in the fibrillating atria of two different kinds (11) They named them L (large) and M (minute) activity and they believed the L waves to be the *f* waves on the regular ECG Prinzmetal et al used a high speed cinematographic technique Their results with respect to *f* waves were not further discussed

In 1956 Osol found the voltage of the *f* waves to be greater in patients with RHD than in pa tients with ASHD (9)

Thurmann and Janney separated the *f* waves into fine and coarse (16) Most authors have later used this description

Thurmann and Janney found that 87 ° of the patients with coarse *f* waves in lead V_1 had RHD while 88 % of the patients with fine *f* waves had ASHD (16) Peter et al found in RHD coarse *f* waves in 85 and in ASHD in 60 As Peter et al used 1 mm as upper limit for fine *f* waves their result is even more noteworthy

In the present series coarse *f* waves occurred in RHD in 63 % and in ASHD in 70 Results from some different reports are reviewed in Table V

The explanation of the different results is not evident but some factors might be important My group of ASHD consists exclusively of patients with myocardial infarctions As the criteria for



Fig 2 The different types of *f* waves within the different etiologic groupings Hatched part of column denotes number of patients with coarse *f* waves, blank part fine *f* waves and black part inconsistent *f* waves

the diagnosis of ASHD are not given in some earlier reports (4 5 9 13 14 16) the selection of the materials is probably different As I have restricted the diagnosis to cases with old and/or acute myocardial infarction (old 19 acute 29 and both 8) my group might have a more serious disease than the clinical materials previously published Skoulas and Horlick reported a few cases in which deterioration of the heart disease caused an increase of the *f* wave amplitude (13)

Another possible factor of importance might be the different mean age in the materials re ported Aravanis et al found the *f* waves to be age dependent (2) The amplitude decreases with increasing age The explanation of this change of the *f* waves with regard to age is unknown In the present material I did not find a significantly higher mean age in the group with fine *f* waves However the groups are small Most authors have not given the age of their materials and if this



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MURAMIDASE ACTIVITY OF BONE MARROW PLASMA

Studies in Haematologically Normal Individuals and in Granulocytopenic Patients

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Abstract Among the cells of blood and bone marrow muramidase is found solely in neutrophil granulocytes and monocytes With certain provisos which are discussed the extracellular muramidase activity seems to be valuable as an indicator of destruction especially of neutrophils

Muramidase activity was measured in bone marrow plasma and peripheral plasma from fifteen patients with no haematological disorders In all cases the activity of the bone marrow plasma exceeded the activity of plasma from peripheral blood a finding suggestive of intra medullary destruction of myeloid cells under normal conditions. The excess activity in bone marrow plasma was, however not great probably reflecting that this intra medullary death of cells was taking place to a small extent only

The same measurements were carried out in nine patients with granulocytopenia associated with various medical disorders The results obtained indicate that muramidase determinations in blood and bone marrow plasma may be a useful tool in the classification of granulocytopenias in terms of decreased production ineffective production and increased peripheral destruction

Kinetic studies concerned with the evaluation of accelerated destruction of mature neutrophil granulocytes have usually been based on labelling with radioactive diisopropylfluorophosphate (DF³ P) (see review by Boggs (1)) A different approach to the study of neutrophil kinetics is to measure enzymes liberated from disintegrating cells Muramidase (lysozyme) is found amongst the cells of peripheral blood solely in neutrophil granulocytes and monocytes (2) and increased activity in serum has therefore with certain provisos been taken as evidence of accelerated destruction of these cells

Fink and Finch (5) were able to divide their patients with granulocytopenia into two groups one with increased numbers of granulocytes in the bone marrow and elevated muramidase activities in blood serum and one with hypocellular bone marrows and reduced serum muramidase activities Based on sequential serum muramidase studies in rabbits these authors suggested that ineffective granulocytopoiesis may precede complete marrow recovery following injury induced by nitrogen mustard However marrow muramidase activities were not investigated

In a study based upon measurements of the disappearance rates of DF³ P labelled neutrophils (6) it was also found that patients with granulocytopenia could be divided into two groups one with normal and one with rapid disappearance Only the latter group showed elevated muramidase index (serum muramidase activity divided by the number of neutrophil granulocytes and monocytes per μl of blood)

Measurements of serum muramidase activity have also been used in the investigation of the granulocytopenia associated with megaloblastic anaemia due to folic acid or B_1 deficiency (13) It was found that these patients had high serum muramidase activities which reverted to normal following therapy This was taken as evidence of increased turnover of neutrophils and it was suggested that analogous to the ineffective erythropoiesis found in megaloblastic anaemia ineffective granulocytopoiesis might be operative in these patients However muramidase activities in the bone marrow plasma were not examined

Although destruction of myeloid cells within the bone marrow (ineffective granulocytopoiesis) thus has been suggested few studies have been carried out on the bone marrow itself in order to examine this hypothesis Based upon autoradio-

Table I *The normal material average values ±S D of muramidase studies*

Muramidase in blood plasma (μg/ml) (26 cases)	Muramidase index (see Methods) (26 cases)	Muramidase in bone marrow plasma (μg/ml) (15 cases)	Muramidase ratio marrow/blood (15 cases)	LDH ratio marrow/blood (15 cases)
104±53	0 29±0 14	237±107	2 92±1 32	≥9

[a] Student s t test performed on the difference between marrow and blood muramidase activities $p<0\ 001$

graphic studies in two dogs Patt and Maloney (12) postulated the existence of the so-called myelocyte sink in which sixty percent of all normal myelocytes were lost Zuelzer (17) presented evidence of intramedullary retention and death of granulocytes (myelokathexis) in a 10 year old girl whose marrow granulocytes showed unique morphological and functional characteristics In a study of lactate dehydrogenase (LDH) and LDH isoenzymes in blood and bone marrow (7) evidence compatible with intramedullary destruction of granulocytic cells was found in one patient bone marrow plasma showed a striking elevation of the LDH iso nzymes characteristic of neutrophils

In the present study measurements of murami dase activity were made in plasma from bone marrow and peripheral blood in a number of haematologically normal patients in order to establish the normal range of extracellular activity the bone marrow Similar determinations were d out in a limited number of granulocyto penic patients in order to investigate whether such measurements could further the understand ing of the mechanism of granulocytopenia

MATERIAL

Fifteen haematologically normal adults hospitalized for various unrelated disorders, constitute the normal mate rial Specifically they fulfilled the following criteria 1) leukocyte count in the blood between 4000 and 10 000 per μl 2) normal differential count 3) normal bone marrow morphology 4) normal renal function (s rum creatinine concentration below 1 3 mg per 100 ml no proteinuria) and 5) no demonstrable infection In thes patients muramidase activity was measured in plasma from bone marrow and peripheral b ood In addition muramidase activity was measured in blood plasma from eleven patients who also fulfilled the above criteria ex cept that the bone marrow was not examined

Nine patients with granulocytopenia (neutrophil count in the blood below .000 per μl) were investigated (Table II) They all had normal renal function and no infection could be demonstrated In these patients muramidase activity was determined in bone marrow plasma and blood plasma

METHODS

Plasma from bone marrow and peripheral blood was ob tained as previously described with EDTA as anticoag ulant (7) In order to minimize blood admixture the maximal volume aspirated from the marrow was ¹/ ml

Muramidase activity was measured by the lyso-plate method described by Osserman and Lawlor (11) This method is simple and reliable The sample diffuses from a well into a gel which contains a microorganism lysable by muramidase The diameter of the zone of lysis is proportional to the log of concentration of muramidase A standard curve was obtained for each lyso plate en zyme concentration in the sample was related to the hen egg white standard *Micrococcus lysodeikticus* was sus pended in molten 1 agarose (phosphate buffer of pH = 6 2 50 mg dried bacteria per 100 ml) This medium was allowed to solidify in Petri dishes and 25 μl of test or standard solution were pipetted into wells 2 mm in dia meter After about eighteen hours at room temperature the diameters of lysis around the wells were measured For dilutions of the standard, a linear relationship was obtained between log concentration and diameters of lysis within the range of 5–500 μg per ml Serial dilution of the sample ensured that readings were made within this range As enzyme standards were always run to-gether with the test samples the time of development was not critical

The lysodeikticus strain used was supplied by Boeh ringer (Mannheim Germany) This strain was compared to the strain supplied by the American Type Culture Col lection (Rockville Maryland) and the two strains were found to give identical results The muramidase standard was hen egg white muramidase supplied by Sigma Chemical Co (St Louis Missouri) This brand was com pared to that of Worthington Biochemical Corp (Free hold New Jersey) and the activities were found to be identical

In order to relate muramidase activity in blood plasma to the number of circulating cells containing this en zyme the muramidase index (13) was calculated for each patient

Muramidase index

$$= \frac{\text{plasma muramidase activity in } \mu g/ml}{\text{no of circ neutrophils and monocytes}/\mu l} \times 10$$

Table II *Clinical data and experimental results of muramidase studies from nine patients with granulocytopenia*

Pat	Sex	Age (y)	Diagnosis	Treatment	Blood: Neutrophils (/µl)	Blood: Monocytes (/µl)	Bone marrow morphology (myelopoiesis): Quantitative	Bone marrow morphology (myelopoiesis): Qualitative	Muramidase in blood plasma (µg/ml)	Muramidase index (see Methods)	Muramidase in bone marrow plasma (µg/ml)	Muramidase ratio marrow/blood	LDH ratio marrow/blood
C C W	♂	71	Myelofibrosis	—	750	10	Sparse	Normal	110	1 45	95	0 86	6 0
A L H	♂	67	Probable myelofibrosis	Prednisone (30 mg/day) testosterone	1140	0	Sparse	Normal	270	1 89	2 0	1 00	4 2
G J	♂	21	Aplastic anaemia	—	140	30	Nearly empty	Normal	38	2 22	0	0 53	4 0
S L L	♂	66	Early pernicious anaemia (Hb 11 g/100 ml)	—	1650	270	Brisk	Megaloblastic	80	1 56	240	0 86	3 9
K K K	♂	40	Paroxysmal nocturnal haemoglobinuria	Prednisone (60 mg/day) Dianabol R folic acid	615	45	Brisk	Predominance of younger forms	190	2 88	370	1 95	22 7
E E	♀	75	Probable disseminated lupus erythematosus	Prednisone (15 mg/day) chloroquine	15 0	70	Sparse	Normal	180	1 19	340	1 89	>2 0
O C	♀	74	Cirrhosis of the liver	—	12 0	1 0	Normal	Normal	68	0 51	360	5 30	13 7
P M V	♂	16	Chronic idiopathic pancytopenia	Folic acid	625	100	Brisk	Predominance of younger forms	47	0 65	350	7 44	5 6
J P	♀	71	Disseminated lupus erythematosus	(a) May 31 —	1370	0	Brisk	Normal	80	0 61	500	6 25	12 0
				(b) June 10 prednisone (30 mg/day)	3265	144	Brisk	Normal	80	0 23	310	3 88	9 0
				(c) June 21 prednisone ([illegible]0 mg/day)	4925	394	Brisk	Normal	70	0 13	110	1 57	9 0
Normal values									104±53	0 29±0 14	237±107	2 92±1 32	>9 0

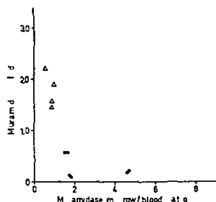


Fig 1 The muramidas index (see Methods) plotted against the marrow/blood muramidase ratio Normal persons are designated ● granulocytopenic patients are divided into three groups △ patients with low O patients with normal and □ patien s with elevated marrow/blood muramidas ratio

The degree of admixture of blood in the bone marrow aspirates was estimated by determinations of LDH activity (analyses performed by the late Dr Th Laursen) in the plasma of the blood and bone marrow samples (7)

RESULTS

The results obtained in the haematologically normal patients are summarized in Table I Average imidase activity in blood plasma was 104± ‹ ug/ml In all these patients muramidase activity in bone marrow plasma was at least 1 5 times that of blood plasma and the average marrow/blood ratio was close to 3

Table II summarizes the results and pertinent clinical data from the nine patients with granulocytopenia It is seen that in four patients muramidase activity in b ood plasma was high although only in one definitely outside the normal range Three patients had nearly normal muramidase indices whereas six patients had abnormally high indices With the exception of one case muramidase activities in bone marrow plasma were within the normal range Four patients had marrow/blood muramidase ratios ≤ 1 and three patients had high ratios

Fig 1 shows the muramidase indices plotted against the marrow/blood muramidase ratios for all th patients examined

Tables I and II also show the ratio marrow/blood for the LDH activities This ratio was higher than the corresponding muramidase ratio in all patients but one (P M V) The high LDH activities in the plasma of the bone marrow aspirates indicate ample representation of marrow

DISCUSSION

Methodological considerations

The applicability of muramidase activity in the plasma of blood and bone marrow as a parameter of neutrophil kinetics rests on the assumption that most of the activity stems from disintegrated neutrophils In the cells of peripheral blood muramidase is found solely in neutrophils and monocytes Muramidase activity in the bone marrow is as could be anticipated demonstrable in all myeloid cells containing neutrophil granules activity increases with cell maturity but little difference is observed in cells more mature than myelocytes (2) However muramidase is found in a number of other tissues and enzyme activity from these may contribute to the activity found in plasma Experiments in dogs in which the granulocyte levels were depressed by means of nitrogen mustard suggested that serum muramidase is derived primarily from neutrophils (5) Similar results were obtained in a study of leukemic patients (14) Other studies have also indicated that serum muramidase activity varies with the neutrophil level (4 9) Still muramidase might be liberated not only from disintegrating but also from intact cells Two investigations militate against this possibility however Firstly no muramidase activity could be demonstrated in saline washings of intact neutrophils (11) Secondly Briggs et al (2) could not demonstrate muramidase liberation from intact cells in smears not until drying had damaged the cells could muramidase activity be demonstrated

Another prerequisite for the application of muramidase activities in the study of neutrophil kinetics is that muramidase activity per mature neutrophil is approximately the same from patient to patient including those with granulocytopenia No information is available on this point

If quantitative estimates of neutrophil kinetics are to be based on plasma muramidase activity knowledge of the rate of catabolism and excretion must be available Such information on muramidase kinetics is not at hand It is known however that impaired renal function affects the

level of blood plasma muramidase activity (8) All patients in the present study had normal serum creatinine concentration and no protein uria

Finally it is not known whether marrow plasma muramidase activity may in part be due to dam age inflicted on the cells during aspiration and centrifugation

In conclusion several aspects of muramidase physiology are not sufficiently well investigated to permit definite conclusions concerning neutrophil kinetics from data on muramidase activity in blood plasma and bone marrow plasma However the available evidence favours the concept that most muramidase activity in plasma stems from disintegrated neutrophils

The normal material

In the present study the muramidase activity in blood plasma of adults without haematological disease was found to be about 100 μg/ml This figure is higher than the activities reported in the literature which gives values between 5 and 20 μg/ml (9 10 14 15) In all these reports how ever the turbidometric method of Smolelis and Hartsell (16) or modifications of this has been used The only published study in which the lyso-plate method used in the present investigation was employed is that of Osserman and Lawlor (11) They found the average muramidase activity in normal persons to be 7 μg/ml when human muramidase prepared by these authors was used as a reference However it was demonstrated that with the lyso-plate method the specific activity (activity per mg) of human muramidase was 8–12 times greater than the specific activity of crystal line hen egg white muramidase (confirmed in our laboratory) When this is taken into consideration the values in the present study are only slightly higher than those of Osserman and Lawlor

In all the haematologically normal persons we have found somewhat higher muramidase ac tivities in marrow plasma than in blood plasma This finding suggests that under normal condi tions a limited number of myeloid cells are de stroyed within the bone marrow The high bone marrow/blood ratio for LDH is in the main due to ineffective erythropoiesis (7) As discussed above quantitative estimates should be made with great caution However our results are hardly compatible with destruction of sixty percent of myelocytes as postulated by Patt and Maloney (12) It is true that destruction of myeloid cells within other cells presumably would not increase extracellular muramidase activity but there is no morphological basis for so extensive phagocytosis of myeloid cells Cronkite et al (3) in their kinetic studies of granulocytopoiesis were also unable to demonstrate a significant loss of cells at the myelocyte level

The patients with granulocytopenia

The diagnosis of granulocytopenia is based on examination of the circulating blood However mature granulocytes are found in several com partments viz a bone marrow pool of consider able size in the blood a circulating and a mar ginated pool and a tissue pool of unknown mag nitude In principle granulocytopenia may de velop as a result of decreased production in effective production a redistribution of pools (e g an increase of the marginated pool on the expense of the circulating pool) accelerated de struction of granulocytes in the periphery or any combination of these

On the basis of measurements of muramidase activity in blood and bone marrow plasma it should be possible to classify granulocytopenia in terms of decreased production (low marrow muramidase activity) ineffective production (high marrow muramidase activity) accelerated de struction in the periphery (high muramidase ac tivity in blood plasma) or combinations of these Even if the small number and the heterogenous composition of the present series of granulocyto-penic patients preclude definite conclusions as to the value of this approach it did seem possible to obtain a grouping of the patients along these lines

The two patients with myelofibrosis (C C W & A L H) and the patient with aplastic anaemia (G J) had low or normal muramidase marrow ac tivities This corresponds well with the hypocellu larity of the marrow and is evidence against signifi cant granulocytoly is in the marrow The murami dase index was elevated in these three patients sug gesting accelerated destruction of neutrophils it should be noted however that the contribution of extramedullary granulocytopoiesis to the mur amidase activity in blood plasma is unknown The patient with early pernicious anaemia (S L L) showed similar muramidase findings which ar

gues against ineffective granulocytopoiesis at this stage of the disease

In two patients one with paroxysmal nocturnal haemoglobinuria (K K K) and one with probable disseminated lupus erythematosus (E E) the elevated muramidase index suggested accelerated peripheral granulocytolysis Muramidase activity in bone marrow plasma was also high so a combination with intramedullary destruction was a possibility However both these patients were treated with prednisone the influence of which on muramidase activity is not established and consequently the interpretation of the findings must be tentative

In three patients one with cirrhosis of the liver (O C) one with chronic idiopathatic pancytopenia (P M V) and one with disseminated lupus erythematosus (J P) the muramidase activity in blood plasma was low and the muramidase index near normal evidence against important acceleration of peripheral destruction of neutrophils In bone marrow on the other hand muramidase activity was high resulting in elevated marrow/blood ratios This picture suggests intramedullary destruction of neutrophils as the cause of the neutropenia and in the patient in whom the findings were most pronounced (P M V) the bone marrow smear showed lively granulocytopoiesis with a predominance of the most immature cells

One pati nt was followed with muramidase measurements before and during treatment with ednisone (Table II) Before treatment the irrow/blood muramidase ratio suggested ineffective granulopoiesis as the prominent cause of granulocytopenia but treatment with prednisone (30 mg daily) for ten days reduced the bone marrow muramidase activity and after treatment for three weeks the marrow/blood muramidase ratio was decidedly low suggesting that prednisone efficiently amended the ineffective granulocytopoiesis in this patient

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EVALUATION OF CHEMICAL AND MICROSCOPICAL METHODS FOR MASS DETECTION OF BACTERIURIA

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Abstract As part of the search for a satisfactory screening test for bacteriuria suitable for mass detection a comparison has been made of the tests that might be useful (Uroscreen TTC test, nitrite–nitrate incubation test, catalase test, and microscopy of urine for bacteriuria and pyuria) and of quantitative urine culture which have been carried out in parallel The results have been compared with the earlier published results obtained by the respective methods.

None of the above screening tests has been found to fulfil the demands that should be placed on a test designed for mass detection As regards the Uroscreen, TTC, and nitrite–nitrate incubation tests and microscopy of the urine for bacteriuria, their sensitivity was good but their specificity unacceptably low Microscopy for pyuria and the catalase test showed too low sensitivity and specificity The nitrite test was the only test with acceptable specificity but its sensitivity was too low to render it acceptable as the only screening method for mass detection Various types of simplified urine-culture methods and Schersten s glucose method remain to be evaluated

The need for simple rapid and inexpensive tests for bacteriuria designed for screening of large populations has increased in recent years since it has been found that asymptomatic bacteriuria often represents an active urinary tract infection in progress or a forerunner of it (17 18 19) At an ordinary medical investigation of a patient a test for bacteriuria can be a valuable complement to the other examinations even though it is not optimally specific and sensitive For mass detection as in health surveys however where as a rule one screening test alone can be made a reliable screening method is essential. It must be highly sensitive and in particular highly specific as persons with false positive tests must be subjected to laborious and costly investigations which will mean an unnecessary load on the organisation of the health survey At present the most reliable method for the detection and quantitation of bacteria in urine is quantitative urinary culture which is generally used for comparison in studies of other methods This method however is time consuming and requires the use of a bacteriological laboratory For screening, simplified cultural methods (5 12 24) and chemical microscopical and electronic methods (33 34) are therefore used

METHODS

In this study the author has tried to evaluate and, in some cases, modify the most common chemical and microscopical screening method and to compare my own results with those obtained by others. The technique of different tests and modifications worked out by the author will be described briefly (*A*) Other methods used will be described under section *B*

A. *Methods for the detection of bacteriuria*

(*a*) Method used for urine culture Urine was cooled to about +4 C and sent in cooled tubes (about +4 C) to the hospital s bacteriological laboratory (Director Prof R. Grubb) where quantitative urine culture was made Bacterial counts above 10^5 per ml were considered to represent bacteriuria, with full recognition of the fact that some of them did not represent true bacteriuria but contamination. The presence of 10^{4-5} organisms per ml of urine was regarded as suspected bacteriuria, particularly if the bladder incubation was short

(*b*) The triphenyl tetrazolium-chloride (TTC) test, devised by Simmons and Williams in 196 (30) is based on the presumption that most pathogenic bacteria in the urine at an alkaline pH reduce water-soluble colourless TTC to insoluble red triphenyl formazan (37) The sample is usually incubated for 4 h in vitro Technique used for the TTC test Simmons and Williams (30) original method The test was read without a concave mirror and centrifugation In case of doubt the precipitate was examined by microscopy

(*c*) Technique used for Uroscreen® test The Uroscreen test which is a commercial variant of the T T C test, was carried out according to the manufacturer s instructions After shaking the samples were incubated for 4 h at 37 C A distinctly red deposit at the bottom of the tube or red coloration of the whole sample was assessed as a positive result In 300 cases the tests were also read after centrifugation for 5 min at 3000 r p m which did not influence the assessment

(*d*) The nitrite method has long been used The most common pathogenic bacteria reduce nitrate to nitrite which normally is not present in the urine and can be readily detected by various colour reagents (6 11 14 23 36) Requirement The presence of nitrate in the urine and a suitable length of incubation The test is simple and rapid The result is independent of the sampling procedure used The technique can be standardized in the following way The urine is incubated in vitro for a suitable period together with nitrate and the nitrite test is then per formed The sensitivity of the nitrite test is thus increased but its specificity will be greatly dependent on the sampling technique The said procedures will make the performance of the test more complicated

Technique used for the nitrite test To 1 ml of urine was added 1 ml of nitrite reagent (31) If distinct red coloration developed within one minute the test was as sessed as positive Other nitrite reagent solutions (?9 32) were used in parallel the results were identical (365 speci ments were tested after storage for one year at − ?0 C)

Technique used for the nitrite nitrate incubation test Four drops of a sterile 10% solution of sodium nitrate in distilled water were added to 2 ml of urine and after incubation for 4 h at 37 C the sample was tested for the presence of nitrite by addition of 2 ml of nitrite reagent solution A distinct red coloration denoted a positive test Weak red coloration was found to represent a bacterial count below 10^5 organisms per ml of urine

The author s modification of the test for the purpose of shortening the incubation period About 10 ml of well mixed urine were centrifuged for 5 min at 3000 r p m The supernatant was decanted away so that about ½ ml remained and 1 drop of a sterile 10% solution of sodium nitrate in distilled water was added The sample was then incubated at 37 C for 15 min before being tested for the presence of nitrite by addition of ½ ml of nitrite reagent solution Distinct red coloration of the sample denoted a positive test

(*e*) The catalase test Most pathogenic bacteria are rich in catalase (peroxidase) an enzyme that is easily demon strable by its ability to break down hydrogen peroxide to water and oxygen Disc flotation Westergren tube and simple test tube methods give approximately the same results (1 9 16)

Technique used for the catalase test Weiser s method (35) was applied Two ml of urine were mixed with 2 ml of 3% H_2O_2 and the sample was then incubated at room temperature for 15 minutes Foam formation on the surface of the fluid or profuse formation of air bubbles denoted a positive test Jarvinen s method (16) was also used in parallel and gave the same result

(*f*) Microscopy of the urine for detection of bacteria Centrifuged and uncentrifuged urine unstained or stained in various ways has been studied with approximately the same results

(*g*) Quantitative and semiquantitative methods for the diagnosis of pyuria as an indirect technique of screening for bacteriuria have been used with divergent results.

Technique for microscopical examination of urinary sediment for bacteriuria and pyuria About 10 ml of freshly passed urine were centrifuged for 5 min at 3000 r p m The supernatant was decanted away so that about 4 drops remained The centrifugate was stained with 1 drop of 10% methylene blue in alcohol and 1 drop was studied under the cover glass at magnification of ×3 0 More than 5 white cells per high power field denoted pyuria. The presence of bacteria in the sediment was graded from 0 to + + + An uncountable number of organisms per high power field (+ + +) denoted a positive test It was often difficult to distinguish cocci from amorphous salts and other debris

Method used for microscopy of uncentrifuged urine 4–5 drops of mixed freshly passed urine were stained with 1 drop of 10% methylene blue in alcohol and 1 drop was then studied under the cover glass at magnifica tion of ×320 The bacterial counts were graded as for the sediment and + + + was considered to denote a positive test It was often easier to distinguish cocci from amorphous salts and other debris in the uncentrifuged than in the centrifuged sample

B *Other methods applied*

(*a*) Measurement of ascorbic acid in the urine To 1 ml of urine was added 0 1 ml of glacial acetic acid 0 9 ml of the mixture was then added to 4 ml of a solution containing 3 3 mg of dichlorphenolindophenol per 100 ml of distilled water At complete decoloration the urine contained more than 10 mg of ascorbic acid per 100 ml (13 15)

(*b*) Measurement of nitrate in the urine One ml of a solution of diphenylamine in concentrated sulphuric acid (10 mg per 100 ml) was added to 1 ml of urine If a blue coloration did not develop in the layer between acid and urine the sample contained less than 0 5 γ nitrate If the test was negative the urine was tested in the same way with a solution of N N diphenylamine in concentrated sulphuric acid (10 mg per 100 ml) if this test was also negative the urine contained less than 0 07 γ nitrate (8) As nitrates and nitramines may give positive results, the possibility that some tests were false-positive as regards nitrate cannot be excluded

(*c*) Measurements of pH Glass electrode (Beckman)

MATERIAL

The studies of the specificity and sensitivity of the above listed methods were made in parallel on the same urinary specimen from

(*a*) 1350 women employed at the County Council hos pitals, whose ages ranged between 16 and 69 years and who attended voluntary health control for uterine cancer Immediately before the gynaecological examination the urine specimens were collected after prior vulval cleans ing with sterile saline solution Those who had abnormal

Table I *Survey of the literature From considerations of space the references are omitted but will be supplied by the author on request*

Method	No of published series	Result of quantitative urine culture			
		> 10⁵ bact/ml urine		< 10⁵ bact/ml urine	
		No of urines	Per cent pos test (range)	No of urines	Per cent pos test (range)
Uroscreen					
Literature	26	3054	78 (27–100)	6 352	5 8 (0–13)
Own series		146	60 2	2 84	20 3
T T C test					
Literature	33	3405	81 (18–100)	15 680	3 7 (0–14)
Own series		93	74 2	1 175	23 1
Nitrite test					
Literature	17	2078	55 (34–90)	13 793	0 6 (0–1 6)
Own series		74	36 5	813	0 5
Nitrite–nitrate incubation test					
Literature	10	1978	79 (51 98)	6 056	6 2 (0–24)
Own series centrifuged		33	81 8	333	4 5
Own series uncentrifuged		37	78 4	348	4 6
Catalase test					
Literature	8	1057	67 (43–100)	953	20 (2–55)
Own series		35	48 6	305	20 3
Pyuria					
Literature	18	1488	69 (32 97)	4 519	19 (3–35)
Own series		1 8	65 6	1 866	21 7
Microscopic bacteriuria					
Literature	16	1472	87 (33–100)	6 167	8 0 (1 2_)
Own series centrifuged		107	70 1	1 440	7 3
Own series uncentrifuged		8	78 6	213	4 7

urine were asked to return for second tests for bacteriuria and other medical examinations The time of sampling was determined by the time of the health control

(*b*) Inpatients and outpatients at the clinic a total of 512 specimens In this series the samples were collected regularly in the morning the patients voided after prior cleansing of the vulva with sterile saline solution

As the results of the tests in the two series did not differ significantly and as it was a matter of a methodological study the two series will be lumped together in the discussion of the results The clinically interesting data on the frequency of renal diseases in hospital personnel will be reported in a following paper

RESULTS

Short survey of the literature

Published results of parallel studies by chemical and microscopical methods and of quantitative urine culture are summarized in Table I The borderline for bacteriuria by culture has been set at 10 organisms per ml of urine Many authors consider however that it should be set at lower counts (7 17 21 22 26 27) Others argue in favour of a higher borderline count (3 20)

Present results

Without any attempt at analysing the causes of the differences in specificity and sensitivity between tests for bacteriuria in different published series (Table I) The author has compared the mean values from these series for each test and the range of published data with his own results The data are recorded as percentages of the number of positive and negative tests respectively in comparison with the results of bacterial culture on the same urine specimen Possible causes of the high proportion of false positive T T C tests in these series were also studied

Table II *Percentage of cases with more than 10 mg of ascorbic acid per 100 ml of urine vitamin supplementation and false positive Uroscreen and TTC tests in different age groups*

Age group (y)	No of examined cases	10 mg of ascorbic acid per 100 ml of urine ( )	Vitamin medication ( )	False positive test Uroscreen ( )	TTC ( )
15–19	131	34 7	13 3	29 2	38 4
20–29	46_	30 0	14 8	20 7	22 7
30–39	261	23 1	16 4	16 6	14 6
40–49	254	16 0	19 2	11 2	12 1
50–69	23?	11 2	15 7	4 8	3 9

A TTC Test and Uroscreen

The TTC test was positive in 69 (74 2%) of 93 urine specimens with more than 10 bacteria per ml *the Uroscreen test was positive in 88* (60 2%) of 146 specimens The result was independent of the type of bacterial flora The proportion of positive TTC tests and Uroscreen tests respectively was as high in the group with urine specimens containing 10^{3-5} bacteria per ml as in the group with 0–10^{3} bacteria per ml The proportion of positive TTC tests and Uroscreen tests for the urine specimens with 0–10 bacteria per ml was 20 3 and 23 1% respectively

As a rule the sensitivity (mean 81 % range 18–100%) and specificity (96 3% range 86–100%) of the TTC test seem to be satisfactory in the published series The corresponding figures for the commercial diagnostic Uroscreen tube which contains the same substance are mean 78 % (range 27–100%) and 94 2 % (range 87–100 %) By prolonging the incubation period some authors have been able to increase the sensitivity but at the same time the specificity was greatly reduced

The proportion of false positive Uroscreen and TTC tests (23 1 % and 20 3 %) in these series is higher than in each of the other series recorded in Table I

(a) *Relation between false positive TTC tests and urinary concentration of ascorbic acid*

A possible factor could be high excretion of ascorbic acid which is a known TTC reducing substance Large numbers of people in Sweden take vitamin preparations regularly during the winter and probably eat much fruit and vegetables

If different ascorbic acid solutions were added to the Uroscreen tubes it was found that a solution of 10 mg per 100 ml of water was required to obtain positive results in all the tubes The urine specimens of 28% of the investigated women and 25% of the urine specimens from the renal clinic contained more than 10 mg of ascorbic acid per 100 ml The ascorbic acid level in the urine was not correlated with unspecific vitamin intake In the series of women both the proportion of urines with more than 10 mg of ascorbic acid per 100 ml and the proportion of false positive Uroscreen and TTC tests decreased markedly with increasing age (see Table II) The proportion of urines with more than 10 mg of ascorbic acid per 100 ml was significantly higher in the group with false positive Uroscreen and TTC tests than in the group that had been assessed as negative in the respective tests (47% and 42 % as against 15% *and 20% $p < 0.0005$ in both cases) In the group* with more than 10 organisms per ml of urine the proportion of urines with more than 10 mg of ascorbic acid per 100 ml was significantly lower than in the others (10 3 % as against 26 2% $p <$ 0 0005)

Accordingly the high proportion of false positive Uroscreen and TTC tests seems to have been due mainly to the presence of ascorbic acid in the urine specimens (10 38) If all the urines with more than 10 mg of ascorbic acid per 100 ml had been regarded as Uroscreen and TTC negative the number of false positive tests would have been reduced to about half and the sensitivity would have decreased by only about 10

The low proportion of urines with more than 10 mg of ascorbic acid per 100 ml in the group with more than 10 bacteria per ml is difficult to explain Among other factors it is possible that ascorbic acid inhibits bacterial growth in urine that ascorbic acid is metabolized by the bacteria that renal excretion is disturbed in pyelonephritis or that the ascorbic acid consumption is high in infections of the kidneys and urinary tract

(b) *Relation between urinary pH and false positive TTC and Uroscreen tests*

The proportion of urines of pH below 6 5 was significantly higher in the group with false positive Uroscreen and TTC tests than in that with negative tests (95% and 94% respectively as against 83 % and 82 $p < 0.001$)

The pH of the Uroscreen tubes after incubation was measured in 533 cases and ranged between 5 and 9 In most of them (88 /) pH was between 7 0 and 8 0 The proportion of false positive tests increased markedly with falling pH Among 71 false positive Uroscreen tests the pH of the Uroscreen tube was below 7 0 in 25 o as against 10 % of 462 negative Uroscreen tests ($p <$ 0 005)

As T T C is most easily reduced at an alkaline pH it was surprising that the proportion of urines with pH below 6 5 was significantly higher in the group with false positive Uroscreen and T T C tests Since the acidity was not correlated with the ascorbic acid level in the urine the acid urine must have contained other T T C reducing substances

The optimum range of pH for the bacterial reduction of T T C is 7 0–8 0 To ensure this range of pH Uroscreen and T T C are buffered with Na HPO$_4$ In the series of women the buffer capacity of the Uroscreen was exceeded in 12 ° of the specimens which could give rise both to false positive and to false negative results The fact that most of the specimens consisted of concentrated morning urine can possibly explain why the buffer capacity was exceeded in such a high percentage

(c) *Relation between other factors and false positive Uroscreen and T T C tests*

Other conceivable causes of false positive Uroscreen and T T C tests may have been contamination with apathogenic T T C reducing bacteria which do not grow on ordinary culture media and the presence of other T T C reducing substances such as glutathione cysteine ketones and urobilinogen in the urine (38) False negative Uroscreen and T T C tests can be explained for instance by impaired T T C reducing ability in some bacterial strains or the presence of factors that have an inhibitory effect on bacterial metabolism A series of Uroscreen batches showed some variations in quality (cf (4)) In 771 cases two parallel Uroscreen tests were performed with tubes from different batches the results differed in 37 cases (5)

Conclusions

The T T C and Uroscreen tests showed too low specificity to make them suitable for mass detection of bacteriuria The high proportion of false positive Uroscreen tests in the present two series seems to be due mainly to the presence of excessive amounts of ascorbic acid in the examined urines

B *Nitrite Test*

As was expected the nitrite test showed low sensitivity Only 27 (36 5 %) of 74 urine specimens with more than 10 bacteria per ml were positive Out of 813 urine specimens with 0–10 bacteria per ml 4 (0 5 o) were positive two of these had 10^{3-5} bacteria per ml The sensitivity was not any higher when the calculation included only urines with more than 10 bacteria of the same species per ml by two consecutive cultures The results agree well with published data on high specificity (mean 99 4 / range 96 4–100 o) and low sensitivity (mean 55 % range 34–100 °) The low sensitivity may be due among other factors to deficient nitrate reducing ability of some bacteria too short incubation in the bladder absence of nitrate in the urine or the presence of factors that inhibit bacterial metabolism Too long incubation in the bladder may also lead to negative results as the nitrate supply is often limited and the formed nitrite is gradually broken down to ammonia and thus disappears

The urinary level of nitrate was measured in 646 cases from the two series At least 18 6 % had less than 0 5 γ nitrate per ml and at least 4 5 o had less than 0 07 γ nitrate per ml of urine

Nitrite concentrations below 0 1 mg per 100 ml cannot be demonstrated with the reagent solution by the method of Smith et al At least 0 13 mg of nitrate per 100 ml is required for the method to be workable In 18 6 of the tested urines the supply of nitrate was insufficient If the investigated persons had been told to eat vegetables or to take nitrate in some other form on the day before examination it might have been possible to increase urinary nitrate and thus perhaps to increase the sensitivity of the method (25) False positive results by the nitrite method are naturally rare Possible explanations of false positive results are contamination with nitrite failure of bacterial culture or growth of L forms which cannot be demonstrated by ordinary cultural procedures In one case in which the nitrite test was positive despite repeated negative urine cultures investiga

tion showed X ray evidence of extensive pyelonephritis changes

C *Nitrate nitrite Incubation Test*

As was expected the nitrate nitrite incubation test showed higher sensitivity (78 4 as against 36 5%) and lower specificity than did the nitrite test Sixteen (4 6%) of 348 urine specimens with 0–10 bacteria per ml were positive including six with 10^{3-5} bacteria per ml The sensitivity would probably have increased further if incubation had been prolonged but then the specificity would have been reduced further Nitrate reducing contaminating bacteria are probably the cause of the false positive results and therefore the specificity will be highly dependent on the sampling technique The results did not differ from those of earlier authors who report a mean sensitivity of 79% (range 51–98%) and a mean specificity of 93 8% (range 76 5–100 %)

Modified nitrate nitrite incubation test

Out of 33 urines with more than 10^5 bacteria per ml 27 (81 8%) were positive and out of 333 urines with less than 10 bacteria per ml 15 (4 5%) were positive six of the latter had a bacterial count of 10^{3-5} bacteria per ml

Conclusion

The sensitivity of the nitrate nitrite incubation test is good but its specificity is highly dependent on the sampling technique

D *Catalase Test*

The catalase test was performed in the clinical eries alone and showed relatively low sensitivity 17 (48 6%) of 35 urine specimens with a bacterial count above 10^5 organisms per ml were positive As could be expected in view of the fact that the cases were selected in regard to renal and urinary tract diseases the catalase test showed low specificity for bacteriuria (72%) Sixty two (20 3%) of 305 urine specimens with less than 10 organisms per ml were positive All the false positive results could be explained by pyuria haematuria or active renal disease As a screening test for renal and urinary tract diseases the catalase test thus showed high specificity (cf (2))

It is often difficult to grade the test because of variations in the size of the air bubbles and the degree of foam formation in different urines According to earlier reports the sensitivity of the catalase test is relatively good (mean 67% range 43–100%) but its specificity low (mean 79 7% range 45–98%) because for one thing red and white cells and the cells of the renal tubules are rich in catalase

Conclusion

For mass detection of bacteriuria the sensitivity and in particular the specificity of the catalase test are unacceptably low The method could possibly be suitable for screening of renal and urinary tract diseases

E *Microscopy of Centrifuged and Uncentrifuged Urine for Detection of Bacteriuria*

Of 1440 centrifuged and 213 uncentrifuged urines with 0–10 bacteria per ml 7 3% and 4 7% respectively were assessed as positive The corresponding figures for the group with 10^{3-5} bacteria per ml of urine were 20 9% and 6 2% The sensitivity was relatively good out of 107 centrifuged and 28 uncentrifuged urine specimens with more than 10 bacteria per ml 75 (70 1%) and 22 (78 6%) were assessed as positive

The low specificity was due mainly to the difficulties of distinguishing amorphous salts and other debris from cocci and of differentiating the group with 10^{3-5} bacteria per ml and apathogenic bacteria from the group with more than 10 pathogenic bacteria per ml Some of these difficulties could probably have been avoided by gram staining but then the method would lose its simplicity and speed

According to earlier reports the sensitivity of the method of microscopy of urine for bacteriuria is high (mean 87% range 33–100 %) but its specificity low (mean 78% range 78–99 %) As the method is subjective individual variations in the estimation of the bacterial count are to be expected

Conclusion

The sensitivity of microscopy of the urine for the detection of bacteriuria is good but its specificity is unacceptably low

F *Microscopy of Urinary Sediment for the Presence of Pyuria as a Sign of Bacteriuria*

This method showed a sensitivity of 65 6 % and a specificity of 78 3 % With a higher borderline for pyuria the specificity would have increased greatly while the sensitivity would have become unacceptably low

The sensitivity and specificity of the method are relatively low in most published series (mean 69 % range 32–92 / and mean 80 8 % range 65–97 respectively) as asymptomatic bacteriuria may progress without pyuria and vice versa The great discrepancy between published results is probably due to differences in the definition of pyuria and to different technique sampling procedure and composition of the material

Conclusion

The demonstration of pyuria is not a suitable screening method for mass detection of bacteriuria because of its low sensitivity and specificity

THE PRACTICAL IMPORTANCE OF A RELIABLE TEST FOR MASS DETECTION OF BACTERIURIA

Table III shows an attempt at illustrating hypothetically the practical consequences that would result from the unreliability of the described methods when used in screening of an assumed population of 100 000 that is about the number of persons whose urines are tested per year at mass examinations in the County Council district of Malmohus The total population of the district 400 000 is examined once every 4-year period With an assumed incidence of bacteriuria of 3 % which is probably too low 390–1350 cases of bacteriuria could be missed every year and 582–19 400 cases could be wrongly diagnosed as bacteriuria and subjected to thorough investigation for urinary tract infection every year

THE NEED FOR IMPROVED METHODS FOR MASS DETECTION OF BACTERIURIA

Summarizing it seems that none of the applied methods can replace quantitative urine culture if reasonable demands are placed on reliability But the fact that most authors have worked with selected materials renders the evaluation difficult

Table III *Hypothetical consequences of mass screening of a population of 100 000 with an incidence of bacteriuria of 3 %*

Method	No of missed cases	No of false positive
Uroscreen	660	5 626
T T C test	570	3 589
Nitrite test	1350	582
Nitrite n trate incubation	630	6 014
Catalase test	990	19 400
Microscopy for bacteriuria	390	7 760
Pyuria	930	18 430

It should be emphasized that in assessing the results obtained by the methods described here the technique of sampling and the length of bladder incubation must always be taken into consideration The same applies to quantitative urine culture The demands for correct sampling technique and suitable length of bladder incubation can never be fully satisfied at mass examinations and worse results than those reported here are therefore to be expected

As tests for bacteriuria have been planned for the new 4-year period of mass examination which will start in 1970 it has been considered necessary to make a new study for evaluation of various tests for bacteriuria under conditions similar to those of a mass examination Schersten s glucose method (28) and some form of simplified cultural method are planned to be included in this study Like the others these methods cannot be expected to reach the required sensitivity and specificity With Schersten s glucose method the cooperation of the examined subjects is necessary as some fasting and incubation of the urine in the bladder are required. This cooperation is certainly difficult to attain in mass examinations The specificity of urine cultures is entirely dependent upon the sampling technique which cannot be optimal in these examinations It is possible however that repeated and/or combined tests may eliminate the false positive results at reasonable cost and amount of work

There are reasons to expect that a method will be found which is based on the demonstration of a specific bacterial metabolite in urine containing bacteria and which is simple to apply and does not require any type of cooperation on the part of the investigated person

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A COMPARISON BETWEEN ALBUSTIX HEMA-COMBISTIX LABSTIX THE SULPHOSALICYLIC ACID TEST HELLER S NITRIC ACID TEST AND A BIURET METHOD

Diagnosis of Proteinuria

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Abstract A comparison between the different tests for proteinuria—Albustix Hema-Combistix Labstix the sulphosalicylic acid test and Heller s nitric acid test—showed unexpectedly great discrepancies.

1 Albustix and Hema-Combistix gave identical results in only 17 of the tested urines The Hema-Combistix readings were higher than the Albustix readings in 81 % and lower in 2

2 Albustix and Labstix showed better agreement the results being identical in 39% The Labstix readings tended to be higher in the lower part of the chart and lower in the upper part of the chart

3 Albustix 2+ to 4+ corresponded in 80 and Albustix (+) or 1+ in ?4% to clearly positive sulphosalicylic acid tests.

4 Albustix 2+ to 4+ corresponded in 91% Albustix 1+ in 53 and Albustix (+) in 15% to clearly positive results by Heller s test

The results obtained with the reagent strip tests proved to depend upon the pH of the urine A comparison between these methods and the biuret method also showed great discrepancies Seventy two per cent of the urines containing more than 39 mg protein per 100 ml and 7% of those with lower protein concentration read 2+ to 4+ with Albustix The corresponding figures for Hema-Combistix and Labstix were 90 % and 26 respectively and 88 and 9 respectively Of the urines containing more than 39 mg protein per 100 ml 88 % were positive by the sulphosalicylic acid test and 89% were positive by Heller s test Of the urines with lower protein concentration (less than 40 mg per 100 ml) 19 and 17% respectively were positive by the sulphosalicylic acid test and by Heller s test The results are discussed and compared with those obtained by other authors The need for more reliable and more objective methods for the detection of proteinuria is emphasized

Since the Albustix strip was introduced in 1957 two combined strips have become available namely Hema Combistix and Labstix (Ames & Co Division of Miles Laboratories Ltd Stoke Poge Slough Bucks England) which indicate not only proteinuria but also haematuria glucosuria and urinary pH Labstix has also an indicator range for ketone bodies The use of the strips has become increasingly popular owing to the simplicity of the technique We have compared the results of protein estimations obtained with Albustix Hema Combistix and Labstix by the sulphosalicylic acid test Heller s nitric acid test and a biuret method

MATERIAL

The tested specimens consisted of fresh morning urine voided after careful cleansing with sterile isotonic saline solution Most of the specimens represented morning urine from women

METHODS

Albustix Hema Combistix Labstix

The maker s instructions were followed strictly The strips were dipped in the urine and taken out immediately superfluous urine was wiped off against the side of the vessel. The indicator range for protein was then within 10 sec compared with the colour chart on the package for each test, which had six gradings 0 (+) 1+ 2+ 3+ and 4+ Reading was done in daylight and by the same observer who had normal colour vision

Heller s nitric-acid test

To a tapering glass were added about [illegible] ml of concentrated HNO_3. About 2 ml of filtered urine were stratified over the acid A weak precipitation in the layer between acid and urine observable only against a dark background was estimated as trace A distinct to marked precipitation was assessed as positive reaction

The sulphosalicylic-acid test

Two drops of [illegible]0 % sulphosalicylic acid were mixed with two drops of urine on a mirror face A faint opalescence

Table I *Biuret—Albustix*

Biuret protein concentration (mg/100 ml)	No of examined urines	Albustix readings					
		Neg	(+)	1+	2+	3+	4+
≤19	129	50	50	23	6	—	—
20–39	47	15	14	11	6	1	—
40–79	34	1	9	9	13	2	—
80–149	25	—	1	6	8	10	—
150–249	26	—	—	2	4	17	3
250–349	27	—	—	—	3	22	2
>349	27	—	1	—	—	13	14
Total	315	66	75	51	40	65	19

was assessed as 'trace' and a distinct to marked precipitation as positive reaction

Estimation of urinary protein concentration by a biuret method

Four ml of urine were mixed with 4 ml of 20% trichloroacetic acid solution The precipitation was centrifuged down (5000 r p m for 15 min) The supernatant was poured off and the sides of the tubes were wiped with a clean filter paper The sediment was stirred into 4 ml of biuret solution by means of a glass rod The specimens were then kept in the dark for about 2 h before reading was done spectrophotometrically at 545 mμ Horse serum the protein concentration of which had been determined by Kjelldahl's technique was used as standard

pH

Glass electrode (Beckman)

RESULTS

Albustix—biuret (Table I)

Out of the urines with a protein concentration of 40 mg or more per 100 ml 79% read 2+ to 4+ with Albustix 13% read 1+ and 7% (+) Seven per cent of the urines with a protein concentration less than 40 mg read 2+ or 3+ 58% of them had more that 19 mg of protein

Table II *Biuret—Hema Combistix*

Biuret protein concentration (mg/100 ml)	No of examined urines	Hema Combistix readings					
		Neg	(+)	1+	2+	3+	4+
≤19	131	4	53	43	24	7	—
20–39	51	1	16	17	13	4	—
40–79	29	—	1	8	13	7	—
80–149	24	—	—	2	8	12	2
150–249	35	—	—	—	2	21	12
250–349	17	—	—	—	—	3	14
>349	27	—	1	—	1	3	23
Total	314	5	71	70	61	57	51

Table III *Biuret—Labstix*

Biuret protein concentration (mg/100 ml)	No of examined urines	Labstix readings					
		Neg.	(+)	1+	2+	3+	4+
≤19	90	9	58	17	5	1	—
0–39	58	5	40	6	6	1	—
40–79	22	—	2	6	12	2	—
80–149	12	—	—	—	10	2	—
150–249	18	—	—	1	4	12	1
250–349	13	—	—	—	6	7	—
>349	21	—	1	—	2	9	9
Total	234	14	100	30	45	34	10

Hema Combistix—biuret (Table II)

Hema Combistix proved to be more sensitive than Albustix Of the urines that had 40 mg of protein or more 90% read 2+ to 4+ with Hema Combistix 8% read 1+ and 2% read (+) Twenty six per cent of the urines with a protein concentration less than 40 mg also read 2+ to 4+ with Hema Combistix 35% of them had more than 19 mg of protein per 100 ml

Labstix—biuret (Table III)

Labstix was also found to be more sensitive than Albustix Of the urines containing 40 mg of protein or more 88% read 2+ to 4+ with Labstix 8% read 1+ and 0 4 read (+) Nine per cent of the urines with less than 40 mg of protein read 2+ or 3+ in 54% of these the protein concentration exceeded 19 mg

None of the indicator methods allowed a more approximate gradation of the protein concentrations over 40 mg per 100 ml

Table IV *Biuret—Heller's test sulphosalicylic acid test*

Biuret protein concentration (mg/100 ml)	No of examined urines	Heller's test			No of examined urines	Sulphosalicylic acid test		
		Neg	(+)	1+		Neg	( )	1+
19	109	78	22	9	102	67	8	17
0–39	46	10	19	17	48	19	13	16
40–79	5	—	8	17	8	7	7	14
80–149	19	—	4	15	5	—	2	3
150–249	29	—	—	29	39	—	—	39
250–349	14	—	—	14	15	—	—	15
349	24	—	—	4	30	—	—	30
Total	66	88	53	175	287	88	50	149

The sulphosalicylic-acid test (Table IV)

Of the urines that had 40 mg of protein or more 88 were assessed as clearly positive and 7° as trace Of the urines with less than 40 mg of protein 19 were assessed as clearly positive and 27 as trace by the sulphosalicylic acid test in 57% and 32% respectively of these urines the protein concentration was more than 19 mg

Heller s test—biuret (Table IV)

Heller s test gave roughly the same result as the sulphosalicylic acid test in relation to the biuret method Of the urines with a protein concentration of 40 mg or more 89 were assessed as clearly positive and 11 as trace Of the urines with less than 40 mg of protein 17 were assessed as clearly positive and 26% as trace in 65° and 46 respectively of these urines the protein concentration exceeded 19 mg

A urine specimen containing more than 349 mg paraprotein per 100 ml was tested by the different methods The three paper strip methods all showed (+) whereas the sulphosalicylic and Heller s tests gave clearly positive reactions

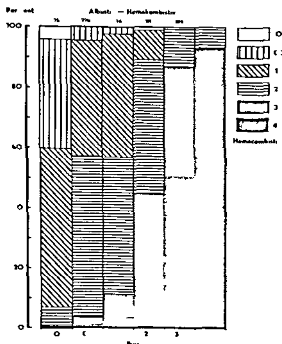


Fig 1 Comparison between Albustix and Hema-Combistix readings of 400 urine specimens.

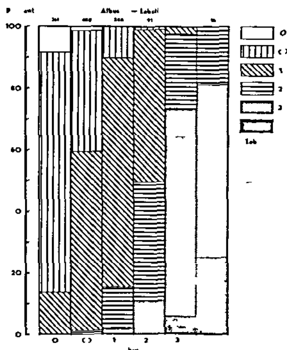


Fig 2 Comparison between Albustix and Labstix readings of 1211 urine specimens

Albustix—Hema Combistix (Fig 1)

Seventeen per cent of the 2400 urine specimens which were tested in parallel with Albustix and Hema-Combistix gave identical results by the two tests according to the maker s charts The Hema Combistix readings were higher than the Albustix readings in 81 and lower in 2% The agreement between the tests increased gradually with increasingly higher readings from 4 % with Albustix 0 to 93 % with Albustix 4+ In 95% Albustix 2+ to 4+ corresponded to Hema-Combistix 2+ to 4+ Of the specimens that read 0 to 1+ on Albustix 61 gave Hema Combistix readings of 2+ to 4+ of these 7 % had been assessed as 0 52 % as (+) and 41% as 1+ on Albustix

Albustix—Labstix (Fig 2)

Of the 1211 specimens that were compared 39 % gave identical readings with Albustix and Labstix according to the maker s charts The Labstix readings were higher than the Albustix readings in 52% and lower in 9° In 75% Albustix 2+ to 4+ corresponded to Labstix 2+ to 4+ The Labstix readings in the lower part of the chart tended to be higher and those in upper part of the

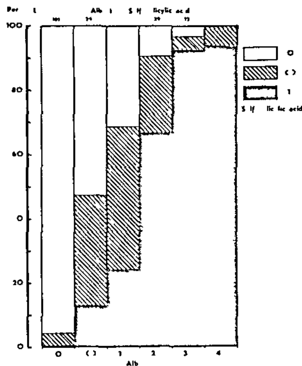


Fig 3 Comparison between results with Albustix and by the sulphosalicylic acid test for 2348 urine specimens

chart lower than the Albustix readings Of the specimens that read 0 to 1+ with Albustix 4 2 o read 2+ or 3+ with Labstix 77 % of these were assessed as 1+ and 23 as (+) wits Albustix

Accordingly the correspondence was much better between Albustix and Labstix than between Albustix and Hema Combistix

Albustix—sulphosalicylic acid test (Fig 3)

2348 urine specimens were tested in parallel by the two methods Of Albustix readings of 2+ to 4+ 80 % corresponded to clearly positive reaction and 14 o to trace by the sulphosalicylic acid test The corresponding figures for readings of 0 or (+) and 1+ were 3 o and 11 o and 24 o and 45 % respectively

Albustix—Heller s test (Fig 4)

1625 urines were tested in parallel with Albustix and by Heller s method Of Albustix readings of 2+ to 4+ 91 ° corresponded to clearly positive reactions and 7 ° to trace by Heller s test Albustix 0 (+) and 1+ corresponded in 15 % 46 % and 32 % respectively to trace and in 1 o 15 % and 53 % respectively to clearly positive reaction by Heller s test Accordingly in comparison with Albustix Heller s test was more sensitive than the sulphosalicylic acid test

Albustix Hema Combistix Labstix and the sulphosalicylic acid test—pH

Of 2233 urines with pH below 6 5 29 % gave Albustix readings of 1+ to 4+ The corresponding figure for 714 urines with pH above 6 5 was 37 o The difference is significant ($p < 0\ 0005$) Of the 1928 and 549 urines with pH below 6 5 13 ° and 18 % respectively read 3+ or 4+ on Hema Combistix and Labstix respectively the corresponding figures for 606 and 191 urines with pH above 6 5 were 21 o and 26 % respectively The difference in the frequency of the urines reading 3+ or 4+ was in both cases significant ($p < 0\ 0005$ and $p < 0\ 025$ respectively) For comparison it may be mentioned that out of 1405 urines with pH below 6 5 21 % were assessed as doubtfully positive and 3 o as clearly positive by the sulphosalicylic acid test the corresponding figures for 943 urines with pH above 6 5 were 21 ° and 2 % respectively Thus there was no difference in the frequency of positive reactions by the sulphosalicylic acid test between urines with low and with high pH



Fig 4 Comparison between the results with Albu tix and by Heller s test for 16_5 urine spec mens

DISCUSSION

There is great need for simple rapid and reliable screening methods for detecting proteinuria The most widely used methods are precipitation with nitric acid precipitation with sulphosalicylic acid and tests which are based on the protein error of indicators As a positive protein reaction usually leads to extensive investigations time-consuming check ups and insurance and employment problems for the patient, it is important that the applied test should not give false positive results On the other hand false negative results may involve the risk that active renal diseases escape treatment and control

Albustix and the protein indicator range of Hema-Combistix and Labstix are all based on the protein error of tetrabromphenol blue The paper strips are impregnated with tetrabromphenol blue and buffered with citrate to pH around 3 5 At alkaline pH the indicator strip changes colour from yellow to greenish blue and it is therefore important that the strip should be well buffered within the acid range

False positive results can be obtained if the strip is kept immersed in the urine sufficiently long for the citrate buffer to be dissolved out False positive results are also not seldom obtained when the urine has high buffer capacity and/or high pH (4 5 8 10 12 15) Extremely low pH values may give false negative results and therefore urine which has been rendered acid should not be tested by these methods Some authors maintain that the physiological variation in pH seldom gives rise to false positive results But in our series a clearly significant difference was noted in the frequency of positive results with the three strip tests between urines at pH above and at pH below 6 5 This difference was not noted with the sulphosalicylic acid test which excludes the possibility that alkaline urines would contain more protein than the acid urines Most of the tested specimens represented morning urines of high specific gravity and probably high buffer capacity Other factors that may account for false positive results are the presence of quaternary ammonium compounds and unphysiologically high urea concentrations

The amino groups in the proteins bind easily with tetrabromphenol blue thus causing a colour change Albumin has more amino groups than have globulins and mucoproteins Free (5) found that the development of the same colour change as that caused by a certain albumin concentration required a globulin concentration twice as high and a mucoprotein concentration 33 times as high Ten times the normal mucoprotein concentration was required to cause a colour change on Albustix Mucoproteins often occur in clumps in the urine however and should these attach to the strip they might easily give rise to false positive results The indicator range will then often be coloured in patches Any admixture of fluor or gland secretion to the urine should therefore be avoided in testing by these methods These sources of error will probably be reduced if the urine has been centrifuged and filtered before the examination Pyuria and bacteriuria are other possible causes of false positive results (8 11)

The globulin concentration in normal urine is about equal to or twice as high as the albumin concentration (9 13) In proteinuria the different protein components usually increase in parallel but discrepancies occur which may possibly influence the result It is for instance known that strip methods based on the protein error often fail to detect paraproteins and are thus less suitable as screening procedures (2 7 17) Our series included one patient who had more than 349 mg paraprotein per 100 ml of urine and in whom strip tests gave readings of (+) throughout, whereas the sulphosalicylic acid and Heller s tests were clearly positive

The sensitivity of Albustix to protein has been studied by many authors Frazer and Baron compared the Albustix results with results obtained by Kjelldahl s method of protein determination and found that protein concentrations as low as 30 mg per 100 ml could be registered with Albustix Free et al (5) and Sherrick and Chertack (15) tested different albumin solutions and found that albumin concentrations of 30 mg and 25 mg per 100 ml respectively could be registered their result was about the same as that obtained by Rennie et al (12) on comparing Albustix readings with immunoassay readings In their tests however Rennie et al were concerned only with the albumin concentration in urine which would mean that a urine containing the same amount of total protein (albumin plus globulin) would probably be negative In our study the protein concentration was estimated by a biuret method which gives relatively uncertain readings at low concentra

tions (1–20 mg per 100 ml) Comparisons between protein concentration determined by a biuret method and with Albustix Hema Combistix and Labstix respectively showed great discrepancies notably at protein concentrations over 39 mg per 100 ml No distinct borderline value for concentration was noted as regards urines that were clearly positive by the strip tests Albustix readings of 2+ to 4+ corresponded to a protein concentration of 40 mg or more per 100 ml in 79% For Hema Combistix and Labstix the figures were 90% and 88% respectively Most of the other specimens had been assessed as (+) or 1+ by the different tests Of the urines with lower protein concentration 7% (Albustix) 26% (Hema Combistix) and 9% (Labstix) respectively read 2+ to 4+ in most of these the protein concentration exceeded 19 mg per 100 ml

Partly the explanation of the discrepancies between the biuret and the strip test results could be that the specimens were neither centrifuged nor filtered before the biuret test and this could have accounted for the falsely too high values obtained in pyuria cases The quantitation of protein concentrations exceeding 30–40 mg per 100 ml by means of the different strip tests proved to be unreliable as has also been found by others (1 8 12)

The gradation of the colour change for the indicator tests varies between different authors Frazer graded the results as negative" trace and positive" MacGregor as protein free some protein present and much protein present and Rennie et al used the categories nil" "trace" intermediate and heavy Most other authors use the maker s colour chart in grading the results To ensure comparable results it is of course best to compare the colour change with a standardized chart Initially the maker s colour chart had only five grades but recently one more block was introduced in the lower part of the chart and this renders comparison with earlier studies difficult

One advantage of the strip test methods over the precipitation methods is that turbidity in the urine does not influence the results

The lighting conditions and the observer s colour vision may influence the reading of the indicator tests

As regards the value of the Albustix method opinions differ Free et al (5) Baron and Newman (1) Frazer (4) MacGregor (10) Huntsman and Liddell (8) Watson (18) Sherrick and Chertack (15) and Rennie et al (12) credit it with high specificity and sensitivity whereas Clough and Reah (2) considering that the test is not sufficiently specific and sensitive believes that urines should be tested for protein by means of older and more reliable tests By comparing Albustix with Hema Combistix and Labstix respectively we found a clear cut difference in the results The two combined strips seldom gave wholly negative results A negative Albustix reading usually corresponded to (+) or 1+ with Hema Combistix and Labstix and Albustix (+) usually corresponded to 1+ or 2+ with Hema Combistix and in about half the cases to 1+ with Labstix With Hema-Combistix there was throughout a tendency to higher readings than with Albustix Labstix on the other hand tended to give higher readings in the lower part of the chart and lower readings in the upper part of the chart in comparison with Albustix Thus the results obtained with Albustix with Hema Combistix and with Labstix were not wholly comparable

The older methods the sulphosalicylic acid test and Heller s nitric acid test have been credited with greater reliability than the paper strip tests But the former also involve sources of error and disadvantages Turbid urine for instance cannot be tested without previous filtering and centrifugation Moreover access to laboratory equipment and chemicals is necessary One advantage is that the tests are positive in paraproteinuria

The sulphosalicylic acid test gives false positive results in the presence of radiopaque materials tolbutamide high penicillin concentrations pyuria and large amounts of mucoproteins or high uric acid levels in the urine The sulphosalicylic acid test has been found to be sensitive to protein concentration as low as 5–30 mg per 100 ml of urine (5 11 16) The variations are probably attributable to the use of different test techniques and methods for protein determination In the present study it was found that 80% of the urines with a protein concentration over 39 mg per 100 ml were clearly positive Nineteen per cent of the urines with lower protein concentration were also positive but 57% of these contained more than 19 mg protein per 100 ml Several comparisons between different types of sulphosalicylic acid and Albustix tests have been published

Huntsman and Liddell (8) found a correspondence of 97 8 o Watson (18) of 93 3° and Sherrick and Chertack (15) of 96 0% Free et al (5) and Rennie et al (12) also found good agreement be twe-n these methods In our study the agreement was poorer Of the urines reading 0 or (+) on Albustix 11 showed trace and 3° were clearly positive by the sulphosalicylic acid test The cor responding figures for urines reading 1+ on Albu stix were 45% and 24% respectively There was good agreement however for the urines reading 2+ to 4+ on Albustix only 6 % being negative by the sulphosalicylic acid test The discrepancies can possibly be explained by the presence of muco proteins and/or pus in some of the urines assessed as negative with Albustix

Heller s nitric acid test may give false positive results in the presence of mucoproteins proteoses urea nitrate high uric acid concentrations resinous bodies radiopaque materials invert soaps iopanoic acid and gallic acids Pigments and homogenates may interfere with the reading

Heller s test is reported to be sensitive to pro tein concentrations as low as 20-30 mg per 100 ml In the present study 89 % of the urines with more than 39 mg protein per 100 ml were assessed as clearly positive Seventeen per cent of the urines with lower protein concentration were also positive in 65 o of these the protein concentration ex ceeded 19 mg per 100 ml Free et al found good agreement between Heller s test and Albustix In the present study there was better correspondence between Heller s test and Albustix than between Albustix and the sulphosalicylic acid test Of the urines reading 0 on Albustix 15 were doubtfully positive and 1 o clearly positive by Heller s test The corresponding figures for the urines with Albustix readings of (+) and 1+ were 46 % and 15% respectively and 32 % and 53° re spectively Of the urines giving Albustix readings of 2+ to 4+ 7 % were doubtfully positive and 91° clearly positive by Heller s test The dis crepancies may possibly be explained by the presence of large amounts of mucoproteins and/ or pus in some of the urines assessed as positive by Heller s test but negative on Albustix

Accordingly none of the applied tests satisfied the demands that should be made on a screening test for proteinuria If more reliable and objective methods are not available the older methods should be used preferably the sulphosalicylic acid test and Heller s nitric acid test It should be possible to screen out some of the false positive results if the tests are repeated However the high frequency of false positive results is a factor to be noted

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IN VIVO DIAGNOSIS OF LOFFLER S ENDOCARDITIS PARIETALIS FIBROPLASTICA

Case Report

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Abstract A clinically and pathologically verified case of endocarditis parietalis fibroplastica Loffler is described. The prominent clinical signs were progressive congestive cardiac failure persistent blood eosinophilia, afebrility The hemodynamic picture simulated constrictive pericarditis On the basis of morphological findings in the initial phase the case might have represented the classical Loffler or the Lennox type of the disease Clinical and pathological considerations are discussed.

During the past decades an increasing number of reports have dealt with several endomyocardial inflammatory and/or degenerative processes of unknown etiology Endocarditis parietalis fibroplastica is of particular interest. According to Loffler s original description in 1936 (20) the disease is characterized by congestive heart failure afebrility significant blood eosinophilia and leukocytosis His cases simulated the clinical picture of constrictive pericarditis but at necropsy the pericardium was found intact pronounced endocardiac thickening in both ventricular cavities was observed however (20 21) Since Löffler s first publication about fifty cases have been reported with good agreement as to the main characteristics of the disease

The endocarditis parietalis fibroplastica is usually a post mortem" diagnosis In the literature reviewed in vivo diagnosis has been reported by Brink and Weber in 1963 and Godeau et al in 1964 (3 11) Numerous reports emphasize that the eosinophilia which is essential for in vivo diagnosis is rarely constant and sometimes may not occur at all during the whole course (3 26 31) Recently Remmele (27)—on the basis of morphological findings—proposed that the disease be divided into different types

CASE REPORT

J V., male aged 41 was admitted to our clinic on 8.9 1967 The only remarkable disease in his previous history was an acute glomerulonephritis at the age of 8 In the middle of 1966 the patient was hospitalized because of general complaints, i e weakness, uncertain muscle and abdominal pains and loss of weight. An increased sedimentation rate and a moderate leukocytosis with 41 per cent eosinophilic granulocytes have been found. No hematologic disease or malignant process could be detected Because of duodenal ulcer a Billroth II type operation was performed on 15 11 1966 Histology showed no malignancy Since his general condition deteriorated, he was readmitted to the hospital with a complaint at this time which already suggested heart failure

On admission to our clinic, deep cyanosis, general edema, bilateral hydrothorax, ascites, faint heart sounds and enlargement of the liver were the principal clinical features. The spleen was not palpable His blood pressure was 105/70 mm Hg, pulse rate about 100 per m n regular The patient was afebrile

Labo atory examinat ons

Urinalysis showed marked proteinuria w th some erythrocytes and hyaline casts in the sed ment ESR was 5 mm h Hematologic in estigations RBC count 4 9 mill. ml, Hb 14.5 g/100 ml, WBC count 10,.00 ml. Differential leukocyte count revealed a 33% eos nophilia and toxic granulation. The eosinophilic cell count va ied between 3080 and 8316 during the whole observation period. Serum proteins, paper electrophoresis, liver function tests showed normal values. Lues serology Mantoux probe serology for ech nococcus and trichinosis were negati e LE-cell phenomenon was also negati e The endogenous creatinine clearance was 45 ml min, which decreased to 13 ml min in the course of the illness, w th con omitant rise of serum



Fig 1 PA chest X ray film

creatinine up to ?6 mg/100 ml The oxygen saturation of the arterial blood was 97.5 The pleural effusion proved to be transudate Histologic examination failed to reveal tumour cells in this or the ascitic fluid A conspicuous ECG finding was the 2 1 atrioventricular block which had developed after a few days of Strophanthine therapy Otherwise the ECG was characterized by repolarization disturbances Fluoroscopy showed signs of pulmonary congestion The horizontally positioned heart was moderately enlarged (Fig 1) Intravenous urography revealed diminished excretion of the contrast material Barium meal showed a partial gastrectomy of Billroth II type otherwise nothing else of note The digital examination of the rectum and rectoscopy were negative The bone marrow analysis showed increased number of plasma cellular and lymphoid reticulum cells as well as eosinophilic myelocytes Polyarteritis nodosa was excluded by muscle biopsy Primary amyloidosis as a possible etiologic factor could not be proved since the rectal mucosa biopsy resulted in normal findings, and the Congo red test—although the results in primary amyloidosis are usually variable and erratic—was normal. The clinical diagnosis of endocarditis parietalis fibroplastica was established on the basis of progressive heart failure and persistent eosinophilia no other reason being found for the latter In order to verify it right heart catheterization and hemodynamic investigations were carried out Because of the patient's poor condition the procedure was confined to a very short time and cineangiography could not be performed The pressure curve of the right ventricle the "dip and plateau formation" implied a considerable reduction of the ventricular compliance The right ventricular end diastolic pressure was 24 mm Hg (Fig) No pulsation could be detected radiokymographically along the contours of the right auricle and those of the apical areas Cardiac output, using the Stewart Hamilton principle and injecting I labelled human serum albumin was 2.15 l min cardiac index 1.33 l/min/sqm and the stroke volume was diminished to 20 ml

The patient was treated by Strophanthine and diuretics (Lasix etha rynic acid) but failed to show a long lasting improvement and the tachycardia hypotension the diminished pulse amplitude and the subjective complaints as well persisted and progressed. Steroids (Prednisolone 30 mg day) were given only in the last two months. During the whole clinical course the patient was afebrile The patient died on 7.12.1967 revealing symptoms of congestive heart failure

The clinical diagnosis was endocarditis parietalis fibroplastica

Pathological findings

At autopsy 3400 ml transparent fluid was found in the peritoneal space 700 ml in the left and 600 ml in the right pleural cavity The heart was of normal size weighing 360 g The pericardium and epicardium were smooth and shiny Adhesions between the two layers of pericardium had not developed The right ventricular wall was hypertrophied The cavity practically disappeared, only the outflow tract was intact The mural endocardium

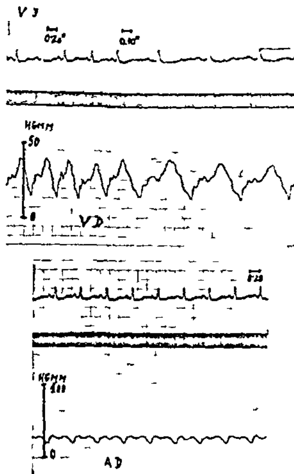


Fig 2 Right ventricular (VD) and auricular (AD) pressure curves.



Fig 3 Hypertrophied left ventricular wall and acute verrucous endocarditis on the mitral valve

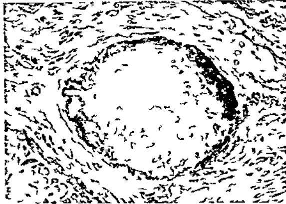


Fig 5 An isolated heart muscle bundle surrounded by rings of increased broken elastic fibers (Weigert s resorcin fuchsin)

was thickened was of grayish white color with decolored thrombi covering it. The left ventricular cavity was reduced to one third the parietal endocardium was also greatly thickened here Its lumen contained a thick massive thrombus The wall of the left ventricle was also hypertrophied with the myocardium replaced by excessive connective tissue On the mitral valve there were minute wartlike excrescences indicating an acute verrucous endocarditis (Fig 3) The kidneys weighed 160 g. Their surfaces were coarsely granulated On section the architecture was still recognizable The macroscopic findings as well confirmed the existence of parietal fibroplastic endocarditis The changes in the kidneys may be ascribed to inflammatory renal disease leading to a secondary contracted kidney

In the histological examinations hematoxylin-eosin a an trichrome combined Weigert s resorcin fuchsin and van Gieson s as well as periodic acid Schiff stainings were used At loupe magnification the scarry thickening of mural endocardium could be well seen and bulky connective tissue streaks penetrated the chamber wall In this scarry connective tissue a mass of increased elastic fibers were found broken into pieces Several, isolated heart muscle groups were surrounded by rings of these elastic fibers (Figs 4 and 5) The changes were predominantly chronic but there were also fields where the mural endocardium had become necrotic slightly swollen with fibrin deposits in it. Vascular changes were found in the medium sized and smaller coronary arteries such as intimal hyalinization and medial hypertrophy The elastic membrane was duplicated and broken into pieces (Fig. 6) There were no eosinophilic leukocytes either in the endocardium or in the myocardium. The spleen showed typical congestive splenomegaly The results of the histological examination of the kidneys was bilateral chronic glomerulonephritis but there were also relatively intact fields Smaller eosinophilic-cell infiltrations were found only in the spleen and in the kidneys. The pathomorphological findings



Fig 4 The cavity of the left ventricle is 3 × 5 mm near the apex Thickening of the mural endocardium. Connective tissue penetrates the chamber wall (combined Weigert stain)



Fig 6 Duplication and breaking of elastic fibers in an arterial wall Intimal hyalinization and recanalization (Weigert s resorcin fuchsin stain)

demonstrated parietal fibroplastic endocarditis developing together with chronic glomerulonephritis

Taking into consideration the clinical course the following should be emphasized

1 The patient was afebrile throughout his illness
2 During the whole observation period i e 1 / years, the eosinophilia was persistent
3 The clinical picture of the disease—as in the cases of Löffler and others (6 11 20 23) simulated constrictive peri arditis The latter was also supported by hemodynamic data
4 Steroid therapy was practically ineffective in this case

DISCUSSION

The development of Loffler s endocarditis can usually be divided into the following phases (25)

The onset of the disease may be acute with abdominal symptoms (6 8 16) or suspicion of respiratory infection (18 32 33) The respiratory distress may lead to an asthmatic state (19) The acute onset may also simulate encephalopathy (10) and even if in most cases the patients are afebrile the disease may begin as an acute febrile illness (24)

The latent beginning is characterized by some general symptoms i e malaise loss of weight uncertain muscle and abdominal pains poly arthritis and polyneuritis (12 22 25) In our case abdominal complaints and muscle pains were the initial symptoms In the early stage the obliga tory morphological findings are the eosinophilic parietal endocarditis interstitial myocarditis and necroses in the myocardium (25) The accom panying eosinophilic pericarditis endarteritis or panarteritis—the latter involving various organs (26)—are possible additional manifestations Often there are no visible changes of the heart but microscopically eosinophilic cellular infiltration of the endomyocardium may be found The endo thelium is swollen necrotizing and small necrotic areas are scattered in the myocardium also In the transitory period of the disease mural thrombi and endomyocardial granulations develop (20 26) Once the thrombi have been organized numerous hemosiderin granula remain at the place of previous inflammation

The fibroplastic degeneration of the parietal endocardium develops in the late phase of Loffler s disease It is accompanied by interstitial and post necrotic scars At this stage when the inflam matory process has subsided blood eosinophilia is not always found (29)

The disease occurs in every age (1 17) It develops most frequently however in the fourth decade The ratio of incidence between males and females is approximately 3 1 (26)

In his recent publication Remmele (27) dis tinguished three types of the cellular form of the *primary acute parietal endocarditis*

(*a*) Endocarditis parietalis eosinophilica with blood and bone marrow eosinophilia and eosinophilic cellular endo-myocardial infiltrates This form strictly represents the original Loffler s disease

(*b*) Endocarditis parietalis neutrophilica charac terized by neutrophilic endo-myocardial cellular infiltrations without blood and bone marrow eosinophilia This type was described by Weber (30) it has not been observed in Europe

(*c*) Endocarditis parietalis neutrophilica with blood and bone marrow eosinophilia and neutrophil cellular infiltrates Lennox (19)

Morphologically in its acute phase our case might have represented either the Loffler or the Lennox type of the disease

Clinically the cardiovascular alterations are the most important They develop either early or only in the last phase of the disease If they occur early the congestive cardiac failure progresses rapidly (10 16 18) while in the case of late development the decompensation takes place more slowly (6 12 20 32) The progressive cardiac failure is mainly the result of the post inflammatory endocardiac fibroplastic degenera tion The enlargement of the heart is not always an obligatory finding In Loffler s original cases the size of the heart was normal On the other hand sometimes marked cardiac dilatation de velops (12 14 32) In our case the heart was moderately enlarged only Ventricular cavities particularly the right one were narrowed to the minimum This may be a good explanation of the stroke volume of 20 ml found The cardiac valves are also often damaged Sometimes they show al terations similar to those of the parietal endo-cardium while in other cases a verrucose valvular endocarditis terminally develops (25) as in this case too Most often mitral insufficiency has been observed but tricuspid incompetence has also been described (33)

Renal involvement may also occur nephritis has been described as a concomitant disease (3 10 27 and present case)

One of the most characteristic signs is the eosinophilia frequently of high degree the cause of which is still unknown In accordance with Gross theory (13) no eosinophilia but eosinophilic cellular infiltration of the tissues may be found in the early and terminal stages of the disease This theory is supported for instance by one of Brink and Weber s cases in which initially and terminally no blood eosinophilia was detectable (3)

According to Loffler the syndrome is a rheumatic symptom complex belonging to allergic diseases Many of the patients exhibited some allergic tendencies such as bronchial asthma (5 19 32) eczema rhinitis vasomotorica (33) In the case of Engfeldt and Zetterstrom (8) and in that of Brink the disease was accompanied by the so called Loffler pneumonia Although most patients showed some allergic manifestations it is striking that quite a few of them had suffered from different chronic diseases which might have a potential pathogenetic significance Tuberculosis (2 5 9 10) malaria (6 12 15) chronic bronchitis (5 18) lues (7 15) and chronic glomerulonephritis (27 and our case) have been found in the patients previous histories Obviously allergic conditions and factors may play a role in the *pathogenesis of the fibroplastic parietal endocarditis* but the final proof of their etiologic significance is still to be found

The outlook of the disease is presently hopeless Its course may be hyperacute or may cover several years Nevertheless when the first signs of cardiac failure or major ECG alterations occur the outcome soon becomes fatal No specific therapy has as yet been developed In recent years some experience has been obtained with steroids (1 3 10 25 28) With moderate doses of Prednisolone a transitory complete remission might be reached but this period is usually very short. On the other hand steroids might be really effective in the early inflammatory stage of the disease At the more advanced stages they proved to be ineffective except for some subjective improvement (1 28 and the present case)

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ACUTE PORPHYRIA IN A HYPERTHYROID PATIENT

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Abstract A report is given on a female patient, 53 years of age with acute porphyria and hyperthyroidism. The coincidence of these two conditions is probably rare but nonetheless presumably more common than is usually believed The diagnosis of hyperthyroidism in acute porphyria may be difficult owing to some clinical phenomena common to both conditions and owing to the fact that in acute porphyria the serum protein bound iodine is often increased The occurrence of hyperthyroidism in a patient with latent porphyria may induce its manifestation and increase the severity of the disease through unknown metabolic pathways

Hyperthyroidism and an acute attack of porphyria have some symptoms and signs in common such as tachycardia elevation of the blood pressure slight fever muscular weakness and psychic and emotional disturbances In the acute phase of both acute intermittent porphyria (AIP) and variegate porphyria the similarity of the clinical manifestations may lead to an erroneous diagnosis of hyperthyroidism Such an interpretation is the more likely to occur since the protein bound iodine in the serum is frequently elevated in AIP (1 6 9) owing to an increase in the binding capacity of the thyroxine globulin (11) The diagnosis of porphyria is not difficult however since it is revealed by an analysis of the urine and faeces for porphyrins and their precursors On the other hand the occurrence of an acute attack of porphyria in a hyperthyroid patient may create real diagnostic and therapeutic problems This combination is rare To the best of our knowledge only one case with AIP and hyperthyroidism has been reported recently (17) and another cited (11) It is possible however that the coincidence of these two conditions is commoner than is usually supposed since although porphyria is a comparatively rare disease hyperthyroidism is relatively common In addition the occurrence of hyperthyroidism in a patient with one of the two commonest types of porphyria AIP or variegate porphyria may have other practical implications since it is quite possible that hyperthyroidism provokes and perhaps prolongs the duration of the acute attacks For these reasons it was considered of interest to give a report on one such patient in whom it was believed that the development of hyperthyroidism did in fact provoke a prolonged attack of a previously unsuspected and asymptomatic variegate porphyria

METHODS

Urinary δ aminolaevulic acid (A.L.A.) and porphobilinogen (Pbg.) were determined according to Mauzerall and Granick (18) and urinary and faecal porphyrins according to Rimington (12) Porphyrin peptid conjugates (X porphyrin) in the faeces were determined by an unpublished method elaborated by Rimington The coproporphyrin isomer distribution in urine and faeces was analysed, using a thin layer chromatography technique (13) which is an adaptation of Eriksen s (5) paper-chromatographic method

The normal values for some of the thyroid function tests used are as follows protein bound iodine in the serum (PBI) 4 0–8 0 μg/100 ml proportionate free thyroxine 0 040–0 070% and absolute free thyroxine 0 49–0 97 × 10⁻ mol/l the triiodothyronin Sephadex uptake 90–115% hydroxyproline in plasma 0 70–1 51 μg ml and the urinary excretion of hydroxyproline 9 7–?3 8 mg/day/m (15)

CASE REPORT

The patient was a woman of 53 with no family history of porphyria. At the age of 38 she gave birth to male non identical twins She had been healthy except for the presence of goitre which was originally observed when she was of school age She never experienced light sensitivity At the age of 47 the patient was suspected of



Fig 1 Patient at admis i n, March 196€

hyperthyroidism and was given a short course of carbi mazole elsewhere Two years later she was more care full studied at the First Department of Medicine Uni ver it of Helsinki At that time she appeared clinically euthyroid and, ex pt for the serum protein-bound iodine (PBI) all laboratory criteria were in conformity with this evaluation The PBI was elevated, however being 10 9 and 14 6 μg 100 ml when determined on two occasions Thyroidectomy for non toxic goitre was sug ested but refused by the patient At that time there was some weakness in the mu cles of the right thumb and X ray examination revealed degenerative changes in some of the cervical intervertebral di cs

At the age of 53 in March 1966 the patient was ad mitted to a local hospital for acute abdominal pains and cons pation. Two days after admission she was given a dose of barbiturates after whi h the urine turned red and she very rapidly developed signs of mental disturb ance and progressing peripheral pareses Markedly ele vated levels of uro and coproporphyrins were found in the urine and the patient was transferred to the Third Department of Med ine University of He sinki

On admission she complained of abdominal pains constipation and inability to void She was weak and prostrated and had almost compl te quadriplegia (Fig 1) She could speak only with difficulty and cou d only slightly move some distal muscles of the limbs. The pulse rate varied between 110 and 130 beats per min the pulse was regular and the ECG revealed only s nus tachycardia The axillary temperature varied between 37 0 and 38 0 C There was a firm multinodular goitre which, on palpation, was estimated to be about 100 g in size Except for a slightly staring appearance there were no specific eye signs present

Attention was paid to the condition of the skin and muscles The skin was pigm nted all over it was con stantly warm and moist and there was palmar erythema The patient appeared flushed There was some muscular wasting in the distal muscles of the extremities in lud ing the inteross i Very marked muscular atrophy was found pro ximally in the shoulder girdle and the gluteal tracts

The diagnosi of porphyria was firmly established on the results obtained from porphyrin analysis of the urine (Table I) Hyperthyroidism was su pected primarily on the basis of som clinical symptoms th flushed appear ance the warmth and mois ness of the skin the palmar erythema and above all the very marked proximal muscle wasting When present muscular atrophy in acute porphyria is mainly of the dis al type By a coincid nce the authors had the opportunity at the sam time to study another patient with acute intermittent porphyria in the acute phase This patient also had tachycardia muscular weakness and nervous symptoms but the skin was cold although slightly moist, and there was no muscular wasting The difference between these two pa tients was quite distinct The laboratory confirmation of the diagno is of hyperthyroidism could not rest solely on the elevation of the PBI, which was 11 9 μg/100 ml, since PBI is frequently elevated in acute porphyria How ever the absolute free thyroxine level of 1.56×10^{-10} mol/l was definitely above the normal range although the proportionate free thyroxine was 0 067 per cent, i e close to the upper normal limit Also the hydroxyprolin in the serum and urine was abnormally e evated in the serum 1.52 μg/ml and in the urinary excretion 78 3 m / day m The serum cholesterol was normal however (265 mg/100 ml) The urinary excretion of creatine was elevated, being 451 mg/day whilst that of creatinine was 650 mg/day The creatine phosphokinase activity in the serum was decreased and that of aldolase normal Own to the condition of the patient no radioactive iodine tests could be carried out. The presence of goitre is apt to direct attention to thyroid function In Finland goitre is endemic however

Other studies will only be briefly mentioned There was a slight decrease in serum sodium (132 mEq) and chloride (9? mEq) whereas s rum potassium, th acid bas balance and serum creatinine were normal The red cell s dimentation rate was 79 mm/h the haemog obin and blood picture were normal Paper electrophoresis revealed a substantial decrease of s rum albumin (2 5 g/ 100 ml) and a slight increas in the alpha globulins. The liver enzymes were normal the intravenous brom sulphthalein test showed a biological half time of 75 min The Widal reaction for Salmonella typhi and S typhimurium was negative and so were tests for thyroid and nuc eic acid antibodies and for rheumatoi l factor There were no L E cells Urinalysis showed slight protein uria a few leucocytes and some Gram negative rods i e a slight pyelonephritis which was treated with anti

Table I *Porphyrins in urine and faeces*

A L A = θ aminolaevuli acid Pbg = porphob linogen Copro = coproporphyrin Uro = uroporphyrin Proto = protoporphyrin

Case	Urine					Faeces (dry weight)			
			Copro			Copro			
	A L A (mg/day)	Pbg (mg day)	(μg/g)	(isomer III % of total)	Uro (μg day)	(μg day)	(isomer III of total)	Proto (μg/g)	X porphyrin (μg/g)
Patient									
April 12 66	19 4	26 2	2420	75	2324				
April 29 66	8 6	2 4	1106	69					
May 5 66	7 1	3 4	1180	92					
August 4 66	4 4	2 7	304	88		489	74	840	
October 1 66	13 0	8 1	940	96		417	81	4?0	
February 2 67	7 0	11 8	1140	94					
January 24 68	8 2	4 1	590	89	10	1205	83	406	275
Patient s sister									
January 68	0 8	1 9	155	87		236		738	99
Patient s son I									
October 66	4 9	3 3	112	84		4 4	31	8 8	
February 67	4 6	2 3	108	86					
Patient s son II									
October 66	5 7	3 4	185	85		132	62	86	
Jan 67	6 4	2 3	197	83					
Normal upper limit (Mean + 2 S D)	3 9	3 4	124	87	30	16 3	55 0	66 6	8

biotics Nothing spectacular was found on X ray examinations except for a slight increase of the heart size and substernal extension of the compressive goitre Scanning of the liver with radioactive Rose Bengal gave normal results

Muscle biopsy from the M deltoideus showed some fibrosis varying diameters of the muscle fibres and small aggregations of lymphocytes i e unspecific changes *Electromyography was postponed until June and by that* time there were unquestionable signs of upper neuron damage and in add tion, some changes indicative of a myogenic component

Treatment was started by giving guanethidine (Ismel n ®) (6) initially 10 mg daily later the dose was increased to 40 mg daily This treatment was started on April 8 and continued until May 20 In addition short courses with prostigmine and chloropromazine were given About two weeks after admission the diagnosis of hyperthyroidism seemed to be established and treatment was started with carbimazole (April 23) in doses of 40 mg daily According to our usual regimen potassium iodide was started at the same time Since an impend ng crisis could not be excluded, large doses (1 0–2 0 g) of KI were given during a few days and the iodide treatment continued with 75 mg daily (5% solution, 15 drops × 3 daily)

Soon after the s art of the guanethid n treatment the constipation changed to diarrhoea. Until the end of May there was no marked change in the patient s condition the pulse rate rema ned between 110 and 130 per min and the temperature was continuously elevated Clinically too the patient made no progress On May 1–2 the serum hydroxyproline was 3 09 μg/ml the urinary excretion 77 6 mg/day/m and the serum cholesterol 214 mg/100 ml The PBI was unreliable owing to administration of iodide At the end of May the cholesterol had decreased to 156 mg/100 ml the serum hydroxyproline was 2 59 μg/ml and the urinary excretion 90 mg/day/m At the end of May there was a deterioration in the patient s condition. The body temperature rose to 39 C and the pulse rate again increased to 140 beats/min An impending crisis was suspected and she was treated with large dos s of iodide and hydrocortisone for a few days during which the temperature decreased rapidly and the general condition improved The iodide treatment was stopped (May 26) and, whereas the carbimazole treatment cont nued potassium perchlorate was also given in daily dos s of 800–1 00 mg. On this regimen there was a marked improvement in the patient s condition during the n xt two weeks and she gradually became euthyroid. At the end of June the PBI was 7 1 μg/100 ml and the serum cholesterol 209 mg/100 ml At the end of July she was entirely euthyro d, the PBI was 6 2 μg/100 ml and the serum cholesterol 51/100 ml She also gradually regained her muscular strength and the muscular atrophy became less marked In July she could mo e by herself after having been bedridden f om late March to the end of June (Fig 2) The perchlorate therapy was stopped at the end of July and the carbimazole treatment continued During a short interruption in the thyro-



Fig 2 Patient after recovery July 1966

static treatment a thyroid scintigram was obtain d which showed a nodular goitre with several hyp rac ive nodu es typical of toxic nodular goitre At that time the PBI was 4 9 μg 100 ml and the serum choles erol 2?6 mg/100 ml During an interruption of four days in the carbima zole treatment the patient was given 20 mC of radioactive iodine The carbimazole was continued for a further three weeks and then finally stopped At the end of September the PBI was 4 0 μg 100 ml and the s rum cholesterol 280 mg 100 ml

The porphyrin excretion pattern is shown in Table I On admission there was a clear elevation of the urinary excretion of A L A Pbg copro and uroporphyrins It is noteworthy that the following analyses showed normal or only slightly elevated Pbg values whereas the urinary excretion of A L A and coproporphyrin remained con stantly elevated Faecal porphyrin analys s were per formed only when the patient was already in remission and they revealed a markedly elevated excretion of coproporphyrin III protoporphyrin and X porphyrin

The patient has been seen on several occasions after treatment she has been steadily improving and has re mained euthyroid In December 1966 the PBI was 7 6 μg/100 ml and the s rum cholesterol 280 mg/100 ml whereas the serum hydroxyproline was still elevated being 2 33 mg/100 ml and the urinary excretion only slightly above the normal upper limit, being 25 8 mg/day/m² The basal metabolic rate was 10

In the summer of 1967 the PBI was 9 0-9 7 μg/100 ml and the serum cholesterol 330 342 mg/100 ml In January 1968 the following values were recorded PBI 9 2 μg/100 ml butanolextractable iodine 7 8 μg/100 ml hydroxyproline in serum 1 38 μg/ml urinary excretion of hydroxyproline 14 5 mg/day/m and the tri iodothyro nine Sephadex uptake 98 She was last seen in April 1968 She was clinically euthyroid and was doing well The PBI was 11 0 μg/100 ml proportionate free thy roxine 0 045% and the absolute level 0 96 × 10 mol/l the tri iodothyronine Sephadex uptake 73 These data are compatible with increas d binding capacity of the binding proteins which however could not be measured at that time

DISCUSSION

The patient had no skin symptoms which are considered typical of the South African cases of variegate porphyria (3) However the biochemical features observed in the present case were typical of this type of porphyria—a relatively slightly elevated and at times even normal urinary excre tion of Pbg associated with a markedly elevated faecal excretion of coproporphyrin III and proto porphyrin (25) This diagnosis was further sup ported by the finding of increased faecal content of porphyrin peptide conjugates (X porphyrin) which according to Rimington et al (23) is a constant abnormality in variegate porphyria No cases of acute porphyria were revealed by the patient s family history In the three relatives of the patient who were examined (sister and two sons) variable patterns of porphyrin excretion were found The patient s sister had a normal urinary excretion of Pbg and A L A slightly elevated urinary coproporphyrin and high faecal porphyrin values Both sons had an increased urinary A L A excretion but only one son showed an increase in the urinary excretion of copropor phyrins and faecal output of copro and proto-porphyrins

In both acute intermittent and variegate por phyria an acute attack is characterized by some clinical signs also commonly found in hyperthy roidism mainly tachycardia nervousness and muscular weakness The symptoms that led to the consideration of hyperthyroidism in the case pre sented were apart from the goitre the patient s flushed appearance the warm and constantly moist skin the palmar erythema and above all the very marked muscular atrophy in the shoulder girdle and the gluteal tracts When present in

acute porphyria the muscle atrophy is usually of the distal type but sometimes muscular wasting also occurs on the trunk (8) In hyperthyroidism when muscular atrophy is evident this is typically found in the proximal muscles and in the shoulder girdle and the gluteal tracts (20) The clinical signs may sometimes be confusing, however and hyperthyroidism should be looked for in some cases of acute porphyria and vice versa Ind ed the patient reported looked very much like a pa tient with chronic thyrotoxic myopathy on the verge of an apathetic crisis (14 29) or the encephalomyopathic crisis of Waldenstrom (27) Real neurog nic palsies are rare in hyperthyroid ism but may occur in the last mentioned condi tion It is the authors firm belief that the hyper thyroidism played a major part in determining the clinical picture but also in the exacerbation of the previously latent porphyria

It is difficult to believe that the simultaneous occurrence of hyperthyroidism and acute por phyria is more than a coincidence It is probable however that such a coincidence is more common than is generally suspected Hyperthyroidism is after all a very common disease and may not be diagnosed when occurring together with acute porphyria Furthermore it is possible that such a combination may in some cases be fatal As pointed out by Mann and deNardo (17) in discuss ing their patient hyperthyroidism may aggrevate the acute porphyria. This can probably be brought about by two mechanisms a) by aggravating the basic metabolic disturbance in acute porphyria and b) by influencing the metabolic changes in the tissue responsible for the clinical signs

In the present case the hyperthyroidism had probably developed gradually during the last few years as it tends to do in toxic nodular goitre in Finland The porphyria attack did not subside until euthyroidism was reached In these circum stances the dose of barbiturates was the factor which finally precipitated the clinical syndrome The site of the metabolic disorder in this type of porphyria is believed to be the liver If so in subjects with a latent metabolic defect, hyper thyroidism may be able to change the enzyme pattern thus increasing the probability that the metabolic derangement will become manifest Hyperthyroidism is known to influence enzymatic processes inhibiting some and stimulating some others (31) and the enzyme patterns in the liver and the function of the liver may also be altered (19) Hence the possibility exists that hyperthy roidism may adversely influence the metabolic changes in acute porphyria Furthermore starva tion has an adverse influence on porphyria (7 12) and in a way hyperthyroidism is in some respects a condition of starvation

Similar reasoning may be applied when discuss ing the effect of hyperthyroidism on the clinical signs and symptoms of acute porphyria Many of the symptoms in acute porphyria simulate sym pathetic overactivity and for instance guane thidine and prostigmine have been reported to be b neficial In hyperthyroidism increased sensitiza tion to the action of catecholamines in the tissues has been suggested and sympath tic blocking agents like guanethidine propranolol and other substances also have an ameliorating effect on many of the clinical manifestations of hyper thyroidism (28) Theoretically some connections may thus exist between hyperthyroidism and acute porphyria as regards the clinical picture and its pathogenesis The mechanism by which the thy roid hormones exert an effect on muscle function is also largely speculative That insufficient pro- duction of high-energy phosphate bonds may be involved is very probable however through un coupling of oxidative phosphorylation chang s in the creatine creatinine metabolism etc (10 21 31) Also pyridoxine deficiency which is known to occur in hyperthyroidism (30) has been im plicated in the genesis of the neurogenic lesion in porphyria (2)

On the other hand the m tabolic ba kground of the neuromuscular lesion of acute porphyria is largely unknown but it seems not to b too far fetched to suggest that such a condition as hyperthyroidism may adversely influence meta bolic derangem nts already present thus aggra vating the clinical manifestations of th lesion It is also well known that some muscular disorders for instance myasthenia gravis and familiar pe riodic paralysis are more common in conne tion with hyperthyroidism (24) In one case of en cephalomyopathic thyrotoxic crisis one of the authors observed a very marked response to prostigmine (16) although the neuromuscular le sion of hyperthyroidism usually does not respond to this drug This suggests that hyperthyroidism was able to creat a pseudo-myasthenic condi tion through some metabolic effects

Since PBI may be elevated in acute porphyria owing to increased binding to thyroxine binding globulin (6 9) the diagnosis of hyperthyroidism cannot be founded on PBI determinations in acute porphyria Other tests which may be helpful in such a situation are for instance the tri iodo thyronine uptake test and the determination of free thyroxine When the binding to TBG rises the T_3 uptake decreases and the proportional free thyroxine as well whereas the absolute free thyroxine level remains fairly normal Additional tests are for instance the glucose 6-phosphate dehydrogenase activity in the red blood cells and the urinary excretion of hydroxyproline (15) The reason why the binding to TBG is increased in acute porphyria has not been clarified It is well known that an increase of TBG is brought about by oestrogens (4) and it has been discussed whether the increase seen in acute porphyria may be due to alterations in the oestrogen metabolism (11)

Mann and deNardo (17) treated their patient with propylthiouracil after considering the possible importance of the similarity in the chemical structure of this compound and the barbiturates The present patient was treated with carbimazole and potassium perchlorate and finally with radio active iodine without adverse effects

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RENAL VASCULAR INVOLVEMENT IN A CASE OF POLYMYALGIA RHEUMATICA WITH TEMPORAL ARTERITIS

A Study of Successive Biopsies

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Abstract A case of polymyalgia rheumatica and temporal arteritis with renal arteriolar involvement is presented Successive renal biopsies before during and after steroid therapy showed an active inflammatory process in the renal arterioles visible as an intense PAS-positive staining of the intimal layer reversible during steroid therapy and succeeded by increasing fibrotic thickening Glomerular histological changes associated with the disease were not present Renal function and urinary findings were normal.

It is suggested that the vascular lesions encountered in this case indicate a disseminating arteritis as the underlying disease process Although renal symptoms and signs in cases of temporal arteritis and polymyalgia rheumatica have been reported no specific vascular lesions reversible by steroid therapy and verified by successive biopsies have to the authors knowledge been described previously

Definite conclusions about the renal vascular lesions in polymyalgia rheumatica and temporal arteritis await final confirmation from larger series with renal biopsies

In recent years a clinical syndrome has been described under a variety of names such as polymyalgia rheumatica (4) polymyalgia arteritica (9) and anarthritic rheumatoid disease (2) which frequently exhibits symptoms from the temporal arteries and may be a disseminating giant cell arteritis related to cranial and temporal arteritis The disease which affects the aged is characterized by acute muscular pain stiffness and pain in different joints without effusion and elevation of the erythrocyte sedimentation rate

Temporal arteritis originally described by Horton et al (12) in 1932 was until recently considered a rarity occurring in elderly people This arteritis is confined to the arteries of the carotid system showing histologically granulomatous inflammatory changes in the media and intima often with giant cells along the disrupted internal elastic lamina and sometimes exhibiting necrotic foci and diffuse fibrotic thickening of the intima with occlusive narrowing of the lumen and thrombosis Recent attention to the extracranial manifestations of temporal arteritis has shown this disease to be rather common with frequent manifestations in the form of polymyalgia rheumatica (5 7 14) and furthermore with involvement of the vascular wall of the aorta and its branches the subclavian and external carotid arteries and coronary iliac splenic and renal arteries (8 11) The other intracranial manifestations of the disease (neurological psychiatric and ophthalmological) have been extensively described by Paulley (15)

The high frequency (30–50) of temporal arteritis occurring in series of polymyalgia rheumatica (1 5 7 10) suggests that temporal arteritis is a local manifestation of a presumably generalized arteritis It has been shown that although temporal arteritis may occasionally constitute a prodromal symptom of polymyalgia rheumatica it frequently complicates its further course occurring 6 to 18 months after the outbreak of the polymyalgic symptoms (14)

Although it is becoming accepted that the disease is primarily a generalized arteritis no conclusive evidence has yet been reported of definite vascular involvement in the renal parenchyma Crosby et al (6) described a case of temporal arteritis with microhaematuria However the patient had recently been subjected to prostatectomy Renal failure in a case of temporal arteritis was described in 1941 by Gilmore (8) The underlying cause was occlusive arteritis of the main renal artery A case of temporal arteritis with acute renal failure in which the causal relationship be

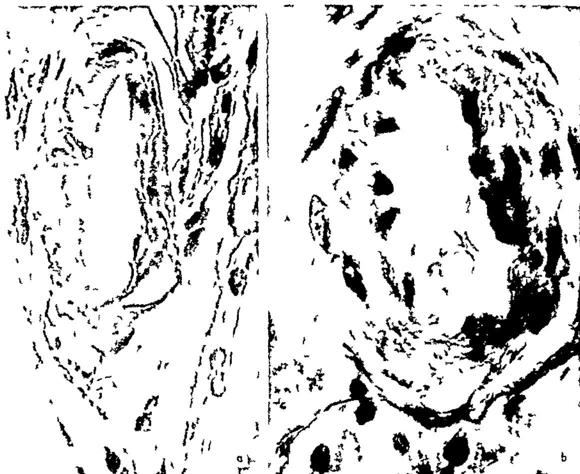


Fig 1 Arteriolar changes during the course of the disease followed by successive renal biopsies Staining PAS (*a b*) × 1700 (*c*) × 1100 (*a*) Before (*b*) during (*c*) after steroid treatment For details see Table II

tween the underlying disease and the complication was difficult to interpret has been described by Balmforth (3) Another case with renal failure and temporal arteritis has been reported by von Knorring et al (13) These two latter cases will be further discussed below

Although there has been awareness of a possible involvement of the renal parenchyma in cases of temporal arteritis the histopathological nature of such a lesion has not been described as yet For this reason a case of typical temporal arteritis complicating the course of polymyalgia rheumatica with clinical symptoms from the kidneys was followed by successive renal biopsies before during and after treatment with corticoid steroids

CASE REPORT

The patient is an 80 year-old female unmarried retired government clerk who in her thirties suffered from gastritis In 1938 partial resection of the stomach was performed because of duodenal ulcer In 1957 elevated blood pressure was recorded (systolic pressure 240 mm Hg) with no signs of congestive heart failure but from 196? on she had been taking digoxine 0 25 mg daily In 1957 an elevated ESR received attention The elevation of ESR persisted and no cause for it could be found From 196? on the patient gradually lost weight approximately 10 kg in four years She has had no history of urinary tract infection

In the summer of 1965 the patient was taken ill with subfebrility pain and stiffness in the shoulderneck region diffuse articular stiffness and muscular weakness occurring elsewhere Two months later she had a constant headache located in the right temporal region

In the following the laboratory parameters essential to the course of the disease are presented separately in Table I During the periods in hospital related below the following laboratory findings were normal the antistreptolysin and antistaphylolysin titres the L E cells test as well as the Waaler Rose and latex tests Immunoelectrophoresis showed unspecific changes of elevated amounts of gamma G immunoglobulins and haptoglobulins The histopatho



logical data obtained during the course of the disease are presented in *Table II*

First admission (October 1965)

The patient was admitted to hospital because of headache elevated ESR, subfebrility malaise and weight loss The records unfortunately contain no account of findings from the temporal arteries during this admission Blood pressure was RR 210/90 mm Hg Heart auscultation revealed a II systolic murmur with maximum in the fifth intercostal space at the left sternal border There were no signs of congestive heart failure Except for left side preponderance the ECG was normal The highest recorded temperature was 37 7 C Repeated urinalysis revealed microhaematuria, slight proteinuria, a normal white cell count and only in one urinary specimen a questionable bacteriuria There were no specific abnormal findings in the excretory urogram Skull X ray studies were normal There was no glucosuria, but a glucose tolerance test was definitely pathological indicating a latent diabetes mellitus requiring no drug therapy The patient received antibacterial treatment after which the urinary sediment was normalized but the elevated ESR remained unchanged. However her polymyalgia rheumatica with temporal arteritis which was probably already in fully active phase escaped notice on this occasion

Second admission (May-June 1966)

After the first hospital admission the patient still complained of muscular and articular discomfort. On three occasions urine controls showed no signs of infection but the ESR was constantly elevated above 66 mm/h In March 1966 the patient herself drew a physician s attention to some tender nodules in her temples The nodules persisted and the patient was finally readmitted to hospital. Her general condition had definitely deteriorated in the interval In the pulseless temporal arteries tender nodules could be palpated The previously mentioned systolic

Table I *Laboratory findings*

Date	ESR (mm/h)	Paper electrophoresis of serum proteins: Total protein (g/100 ml)	Globuline (of total protein): alfa	gamma	Haemo globin (g/100 ml)	Urine: Protein	Cell count in sediment	Serum iron (µg/100 ml)	TIBC (µg/100 ml)
1965 Sept	72–83	6 8–8 3	9 6–11 2	20 3–28 3	11 2	Slightly positive (1/9 specimens)	Red cells on admission (10 control specimens normal)	29	—
1966 May June [a]	7–94	8 0–8.2	8 3–8 6	26 8–27 8	11 2	Negative	Normal	25	288
1966 Sept [b]	52 59	6 9	8 2	27 2	11 7	Negative	Normal	69	303
1967 Sept	5?	—	—	—	11 0	Negative	Normal	39	429
Nov	22	—	—	—	12.6	Negative	Normal	40	410

[a] Prednisolone treatment started June 2 1966
[b] Prednisolone treatment stopped in March 1967 and reinstituted in Sept 1967

Table II *Biopsy findings from the right kidney and temporal artery*

	Renal biopsies Caudal pole of the right kidney
Before treatment June 1966*	Glomeruli tubuli and interstitium histologically normal Intimal PAS positive thickening of several arterioles
During treatment Sept 1966	Tubuli and interstitium normal Glomeruli normal or slightly thi ke ned with doubtful capsular adhesions The PAS positive staining in the arteriolar intima decreased Fibrotic thickening recognizable
After treatment Sept 1967	Two out of six glomeruli completely hyalinized the others normal Tubuli and interstitium normal No PAS positive material recogniz able In reased fibrotic thickening of arterioles

Before treatment June 1966 biopsy of the temporal artery giant-cell arteritis

heart murmur could still be heard and further auscul at on revealed murmurs over both carotid arteries and a stenotic murmur over the right axillary-brachial artery and the abdominal aorta Except in the temporal arteries the pe ripheral palpatory pul e f ndin, were normal The e was no haematuria or proteinuria Renal funct on tests were normal The diabetes was controlled by diet only Aortic arch an iography showed only slight stenosis of the right axillary artery Temporal artery and renal biopsies were performed (for details see Table II)

The disease was now interpreted as a temporal giant cell arteritis with possible systemic involvement of the arteries Steroid treatment was initiated (June 2 1966) with 15 mg predni one daily which gave prompt allevia tion of the subjective symptom and subsidence of fever The patient wa discharged with a maintenance dose of 5 mg prednisone daily

Third admiss on (September 1966)

Since July 1966 the patient had been completely free from the aforementioned symptoms and her general condi tion was greatly improved The temporal arteries had r mained pulseless but nodules and tenderness were no longer found. Because of the patient s hi tory of muscular and joint symptoms and their dramatic response to small dose steroid therapy the disease was interpreted as poly myalgia rheumatica complicated by temporal arteritis with disseminatin, involvement of various arteries A new renal biopsy was performed and the steroid therapy continued with 10 m, predni one every other day for six months

Fourth admission (September 1967)

After the third admission and completion of the steroid therapy the patient was in such good health that she did not return for further examination until she wa con tacted in the autumn of 1967 She had then been without treatment for six months The findings from the temporal arteries were unchanged A third renal biopsy was per formed Ophthalmoscopic findings were normal Because of her constant sideropenic anaemia intramuscular iron therapy was administered but without any remarkable effect The anaemia was concluded to be probably a manifestation of an underlying, presumably immunological disease and steroid therapy was therefore reinstituted (10 mg prednisone every other day) Since discharge from hospital the patient has been seen once and there has been a favourable response to this renewed steroid therapy with normalization of the ESR and elevation of the haemoglobin level.

COMMENTS

The reported case shows all symptoms and signs typical of polymyalgia rheumatica and temporal arteritis as well as histological evidence of giant cell arteritis There were signs of stenotic processes in the right axillary brachial artery the external carotids and the abdominal aorta The nature of these stenotic processes whether arteriosclerotic or arteritic cannot be decided upon from the in formation available The onset of renal complica tion with accidental haematuria focused attention on the possibility of renal involvement Successive renal biopsies gave conclusive evidence of renal vascular lesions located in the intimal layer of the arterioles On staining these arteriolar lesions showed PAS positivity which was reversible in connexion with steroid therapy This seems to exclude an arteriosclerotic or diabetic aetiology of the vascular lesion Fibrotic thickening of som renal arterioles occurred during the course of the disease Although there were histopathological changes in some glomeruli there was no evidence of acute lesions or progressive changes The glomerular findings in some biopsy specimens could better be explained as a patchy senile hyalinizing degeneration as a normal part of the ageing process This interpretation is further sup ported by the fact that in all biopsy sp cimens all or the majority of glomeruli were completely intact Furthermore the sparseness of abnormal urinary findings and normal renal function support the suggestion of a preglomerular vascular lesion only

The previously reported cases of acute renal failure associated with temporal arteritis (3 13) were probably due to pre and postrenal con tributory factors respectively although the above described renal arteriolar vascular lesions were

probably present In Balmforth s (3) case renal failure occurred in close connexion with a cardiac complication with precordial friction rubs systolic hypotonia and acute pulmonary oedema as well as serious local arterial complications (blindness etc) The patient developed oliguria haematuria and proteinuria in spite of uninterrupted prednisone therapy By increasing the steroid dose Balmforth obtained a favourable response in the form of diuresis leading to polyuria and the disappearance of proteinuria and haematuria within a few days The author s suggestion that the renal failure was due to a giant cell arteritis was mainly based upon the favourable response to intensive steroid therapy Oliguria polyuria as a complication associated with prerenal circulatory failure can in our opinion occur without any local disease process in the kidney The findings in our case however seem to indicate that an arteriolar process may have been present concurrently in Balmforth s case and thus increased the susceptibility to ischaemic renal damage The prerenal circulatory failure could also have been an aspect of a generalized arteritis Hence the favourable effect of intensive steroid therapy Renal biopsy was not performed in Balmforth s case In the case reported by von Knorring et al. (13) the renal biopsy was performed during effective steroid therapy and furthermore a renal calculus had just been passed from the same kidney The histological picture also showed signs of a late chronic interstitial process but a specific arterial involvement could no longer be recognized

Our case shows an active inflammatory process in the renal arterioles i.e a PAS-positive staining of the intimal layer reversible during small-dose steroid therapy and succeeded by increasing fibrotic thickening Such a lesion has not to our knowledge previously been described in cases of either polymyalgia rheumatica or temporal arteritis The preceding history typical of polymyalgia rheumatica complicated by temporal arteritis and possible involvement of several large vessels and the renal lesions described would indicate the presence of a disseminated inflammatory process in the whole arterial tree Most authors stress the favourable response to very small steroid doses in cases of polymyalgia rheumatica as well as in temporal arteritis (14) This seems further to argue against the possibility of another type of arteriolar process in the case reported The histological and laboratory findings as well as other clinical data were also negative in respect to some other arterial disease From the standpoint of differential diagnosis polyarteritis nodosa constitutes the main alternative in the case presented but the benign course of the disease and the favourable response to small steroid doses as well as the histological changes recorded seem to exclude this latter alternative as well as an arteriosclerotic or diabetic disease process

We are aware that no definite conclusions about the renal vascular involvement in polymyalgia rheumatica can be drawn from a single case Our findings are suggestive of such an involvement but await final confirmation from a larger series with renal biopsies

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LIPID CHANGES AFTER INCUBATION OF NORMAL PLASMA

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Abstract The individual phospholipids as well as the triglycerides, free fatty acids total cholesterol and its non-esterified fraction have been determined on a normal material comprising 37 men and women of different age groups Young men exhibited significantly higher values of lysolecithin in comparison with elderly men and women irrespective of age

Incubation studies revealed a lecithin splitting activity with subsequent formation of lysolecithin and release of fatty acids. Simultaneously the well known lipase activity was found splitting also the triglycerides and releasing further amounts of free fatty acids Free cholesterol decreased to a significant extent, probably by transesterification of fatty acids released during the incubation from lecithin and probably also to a lesser degree from triglycerides No change of sphingomyelin was noted and phosphatidylethanolamine also remained unchanged during the incubation. The reported lysolecithin increase in incubated plasma probably explains the previously reported reduced sedimentation rate of red cells added to such a plasma The increment of free fatty acids may also contribute to the same result to a lesser extent.

In 1921 Fåhræus (11) published his fundamental work on the suspension stability of blood and stated that the sedimentation rate of red cells diminished when serum was incubated at body temperature for 4–6 h before the cells were added to the sample That means that the suspension stability was increased He was not able at that time to explain these stability changes Bergenhem and Fåhræus (3) in 1936 and Bergenhem in 1938 (2) discussed the matter in more detail and from their experiments they concluded that the incubation probably caused a formation of lysolecithin by the enzymatic splitting of the lecithin molecule Lysolecithin is known as a strongly surface active agent and thus able to bring about hemolysis of red cells or—in lower concentrations—to transform the cells from biconcave discs into spherocytes which are unable to form rouleaux —the prerequisite for an increased sedimentation rate

As a matter of fact Bergenhem and Fåhræus demonstrated the presence in normal serum of a hemolytically active substance with the same solubility properties as lysolecithin which could also be isolated in larger quantities from incubated serum This finding was later confirmed by Singer (18 19) Berlin (4) and Collier and Wilbur (7)

This paper deals with the same problem but by using modern technique an attempt has been made to elucidate further the lipid changes taking place during the incubation of normal plasma

MATERIAL AND METHODS

The material comprises 37 normal men and women, chiefly doctors, medical students and nurses It has been divided into groups according to age and sex as shown in Tables I–III All precautions were taken to avoid in the younger female material the inclusion of early pregnancy cases and consumers of contraceptive pills which are known to affect the phospholipid as well as the triglyceride content of the blood plasma The age distribution within the groups and the means are indicated in the tables Some statistical calculations are also included

Blood samples were drawn in a fasting state in heparinized tubes, cooled in ice water and immediately spun in a refrigerated centrifuge at 4 C

The extraction of lipids and the determination of phospholipids by thin layer chromatography (TLC) were performed in detail as described by Vikrot (?) Determinations of cholesterol and triglycerides were made on aliquots of the same original lipid extract. All phospholipid determinations were made in duplicate with two chromatography applications of each set, other analyses in ordinary duplicate samples.

Total cholesterol was determined by the method of Zlatkis et al (25) as modified by Vikrot. Triglycerides were determined according to Carlson (6) and phosphorus determinations mainly according to Bartlett (1) but slightly modified by Vikrot. Free cholesterol was deter

Table I *Plasma lipid and phospholipid values for different ages and sexes*

FFA = free fatty acids TG = triglycerides TC = total cholesterol FC = free cholesterol TP = total phospholipids LL = lysolecithin SF = sphingomyelin PC = lecithin PE = phosphatidylethanolamine S E M = standard error of the mean *n* = number of subjects For triglycerides the mean was calculated after conversion to logarithms (5) and the range is given

		FFA (mEq/l)	TG (m*M*)	TC (m*M*)	FC (m*M*)	TP (m*M*)	LL (m*M*)	SF (m*M*)	PC (m*M*)	PE (m*M*)
Women 45–63 y	$\bar{x}$	0 53	0 99	7 02	2 03	3 88	0 21	0 84	2 79	0 05
	S E M	0 04	(0 53–1 70)	0 51	0 27	0 19	0 01	0 05	0 13	0 01
	n	9	9	9	9	9	9	9	9	9
Women 20–30 y	$\bar{x}$	0 61	0 71	4 96	1 46	3 23	0 19	0 71	2 29	0 03
	S E M	0 13	(0 36–1 20)	0 34	0 12	0 13	0 01	0 05	0 10	0 01
	n	4	10	10	4	10	10	10	10	10
Men 42–60 y	$\bar{x}$	0 51	1 17	6 29	1 98	3 21	0 21	0 69	2 28	0 05
	S E M	0 05	(0 69–2 22)	0 71	0 38	0 22	0 01	0 05	0 16	0 01
	n	7	8	8	4	8	8	8	8	8
Men 25 37 y	$\bar{x}$	0 39	0 89	5 69	1 57	3 17	0 27	0 65	2 23	0 05
	S E M	0 06	(0 59 1 41)	0 38	0 21	0 08	0 02	0 03	0 06	0 01
	n	5	10	10	7	10	10	10	10	10

mined by the method of Crawford (8) and free fatty acids (FFA) according to Trout et al (21)

All determinations were made on native heparinized plasma and also on plasma incubated for 6 h at 37 C This time was chosen after serial determinations at different lengths of incubation The series showed that th lipid and phospholipid changes started gradually with clearly demonstrable changes already after 2 h reaching a relative plateau level around 6 h incubation After 24 h a moderate further change could be demonstrated

Statistical methods

The statistical calculations were made according to Snedecor (20) The significance of differences between groups was evaluated by the *t* test and changes after incubation by the paired *t* test For triglycerides the mean for various groups was calculated after conversion to logarithms as it has been found that trig yceride values show a log normal distribution (for reference see Carlson (5)) Changes of triglyceride values after incubation were however calculated from the original values

RESULTS

As demonstrated in Table I there is an obvious sex difference in the basic lysolecithin level between young men and young women The former group shows a mean value of 0 27 m*M* lipid phosphorus per litre plasma whereas the latter group is scattered around a mean value of 0 19 m*M* This difference is highly significant ($P <$ 0 001) No sex difference could be ascertained between middle aged or elderly subjects and all female values regardless of age were at approximately the same level as middle aged men

The mean lecithin values show no definite difference between the groups The moderately increased lecithin level in elderly women is probably significant ($P <$ 0 05–$P <$ 0 001 in relation to the other groups) As regards the mean values of sphingomyelin and phosphatidylethanolamine (PE) they w re about 0 72 m*M* and 0 05 m*M* respectively In comparison with the results of earlier investigators the levels of all phospholipid fractions were within the reported range with the exception of the mean PE values of the present study which were definitely lower than reported in the literature

After incubation of the plasma for 6 h a series of phospholipid changes was demonstrated Thus the lysolecithin level increases considerably above the basic value the mean increase on incubation being about 100 % or 0 22 m*M*

Simultaneously a corresponding decreas of the lecithin takes place This applies not only to the mean value of the tabulated age and sex groups but also to every individual series of determinations In addition this decrease of the lecithin is closely related to the increase of the lysolecithin In fact the corresponding values are almost identical Calculations from the figures reported in Table I have shown that lecithin on incubation at 37 C for 6 h can be degraded to lysolecithin at a rate of approximately 0 038 μM/ml/h

The sphingomyelin and PE levels are not altered significantly during incubation the different

Table II *Plasma lipid and phospholipid values after incubation (6 h 37 C)*

Symbols as in Table I

		FFA (mEq/l)	TG (mM)	TC (mM)	FC (mM)	TP (mM)	LL (mM)	SF (mM)	PC (mM)	PE (mM)
Women 45–63 y	$\bar{x}$	0 66	0 91	7 15	1 51	3 90	0 47	0 84	2 53	0 05
	S E M	0 05	(0 69–1 51)	0 52	0 34	0 20	0 01	0 04	0 16	0 01
	n	9	9	9	9	9	9	9	9	9
Women 20–30 y	$\bar{x}$	0 72	0 59	4 89	1 21	3 17	0 39	0 70	2 06	0 04
	S E M	0 13	(0 22–1 10)	0 34	0 09	0 14	0 02	0 05	0 10	0 01
	n	4	10	10	4	10	10	10	10	10
Men 4 –60 y	$\bar{x}$	0 71	1 07	6 19	1 69	3 19	0 42	0 69	2.06	0 05
	S E M	0 08	(0 52–2 08)	0 69	0 39	0 24	0 02	0 06	0 17	0 01
	n	7	8	8	4	8	8	8	8	8
Men 25–37 y	$\bar{x}$	0 52	0 76	5 77	1 16	3 18	0 49	0 67	2.02	0 05
	S E M	0 07	(0 3?–1 19)	0 39	0 16	0 07	0 04	0 03	0 07	0 01
	n	5	10	10	7	10	10	10	10	10

group mean values are seen in Table II as well as the standard error of the means

At the same time other lipid changes occur as shown in Table III The free fatty acids increase on an average 0 14 mM There is a slight decrease of the triglycerides amounting only to 0 10 mM The total cholesterol remains virtually unchanged but the level of free cholesterol decreases to a considerable extent This decrease is in the order of 0 41 mM—calculated on the mean value of the total material

DISCUSSION

During the last decade a renewed interest has arisen in the determination of the individual phospholipids in different body fluids and especially in the blood plasma This evolution has been facilitated very much by the development of TLC on silica gel for differentiation of the phospholipids Thus a number of investigators (9 16 17 22 and others) have reported normal values of the phospholipid fractions based usually on relatively small materials Only occasionally has a sex

Table III *Changes in plasma lipid and phospholipid values after incubation (6 h 37 C)*

P refers to the significance of the difference Other symbols as in Table I
Changes in triglycerides were calculated from the original values

		FFA (mEq/l)	TG (mM)	TC (mM)	FC (mM)	TP (mM)	LL (mM)	SF (mM)	PC (mM)	PE (mM)
Women 45–63 y	$\bar{x}$	+0 13	−0 09	+0 13	−0 52	+0 02	+0 26	−0 01	−0 26	±0 00
	S E M	0 0	0 04	0 11	0 06	0 04	0 01	0 02	0 04	0 01
	n	9	9	9	9	9	9	9	9	9
	P	<0 001	<0 05	NS	<0 001	NS	<0 001	NS	<0 001	NS
Women ?0–30 y	$\bar{x}$	+0 11	−0 12	−0 07	−0 26	−0 06	+0 20	−0 0_	−0 23	±0 00
	S E M	0 0_	0 01	0 07	0 03	0 03	0 0	0 01	0 0	0 00
	n	4	10	10	4	10	10	10	10	10
	P	<0 01	<0 001	NS	<0 01	<0 05	<0 001	NS	<0 001	NS
Men 4?–60 y	$\bar{x}$	+0 20	−0 08	−0 10	−0 29	−0 01	+0 21	±0 00	−0 23	±0 00
	S E M	0 05	0 0	0 09	0 12	0 03	0 0_	0 01	0 03	0 00
	n	7	8	8	4	8	8	8	8	8
	P	<0 01	<0 01	NS	NS	NS	<0 001	NS	<0 001	NS
Men ?5–37 y	$\bar{x}$	+0 12	−0 10	+0 08	−0 41	+0 01	+0 22	−0 0_	−0 21	±0 00
	S E M	0 05	0 05	0 15	0 12	0 03	0 03	0 02	0 05	0 01
	n	5	10	10	7	10	10	10	10	10
	P	NS	<0 05	NS	<0 05	NS	<0 001	NS	<0 001	NS
Total	$\bar{x}$	+0 14	−0 10	+0 01	−0 41	−0 01	+0 22	±0 00	−0 23	±0 00

Table IV *FFA balance after incubation of normal plasma*

FFA released from lecithin mM = F_1
FFA released from triglycerides mM = F_2
FFA used in the esterification of free cholesterol mM = F_3
FFA net accumulation in plasma after incubation mM = F_4
Thus
$F_1 + F_2 \times 3 = F_3 + F_4$ or
$0.23 + 0.10 \quad 3 \simeq 0.41 + 0.14$

differentiation been made Gjone and Orning (12) reported a material of eight men and eight women none over the age of 40 years They found no sex difference with regard to lysolecithin Their lysolecithin values were however about $^1/_3$ lower throughout than the figures reported by earlier investigators and by the present authors From data given in this paper there is obviously a significant sex difference in the lysolecithin values but this holds true only for young people the young men showing definitely *higher levels than women*

To the best of our knowledge only a few more or less systematic incubation studies have been made earlier with regard to the phospholipid and lipid changes (10 13 14 15 23 24) The first mentioned authors (Etienne and Polonovsky 1960) found in long term incubation studies up to 72 h that the lecithin level decreased considerably the lysolecithin increased to a relatively small extent 50 above the original value during the first 48 h of incubation but later exhibited a significant decrease below the *starting level* Glomset (13 14) and Glomset et al (15) using isotopically labelled lecithin and also ultracentrifugal flotation in their experiments on non incubated and incubated plasma found an increment of the lysolecithin on incubation and a corresponding decrease of the lecithin and also a diminution of the unesterified cholesterol From their data they concluded that a transesterification of free cholesterol occurred by a direct transfer of fatty acids from the lecithin This reaction they suggested was accomplished by the action of a fatty acid transferase with the concomitant dissociation of lysolecithin from the reaction site Vogel and Zieve (24) found an increase of lysolecithin on incubation for 4 h of only 10 % On the contrary Vogel and Biermann *(23) were not able to show any signs of lecithinase* activity as measured by lysolecithin determination in their in vitro incubation system of normal plasma and egg yolk lecithin

Actually there occurs a breakdown of lecithin during the incubation with a corresponding increase of lysolecithin Consequently fatty acids are split off from the lecithin molecule As the lecithin content diminishes on an average by 0 23 mM and lysolecithin is formed at the same rate of about 0 22 mM clear evidence is gained that the suggestion of Bergenhem and Fåhræus (3) *that lysolecithin is formed during incubation by* the splitting of lecithin—which assumption they based on relatively inaccurate experimental data —has been proven true

There are some earlier reports on the splitting during incubation also of PE to the corresponding lyso compound This has not been demonstrated in this investigation as the PE values are essentially the same before and after incubation One explanation of this discrepancy may be technical difficulties in distinctly separating the lyso PE spot in the *TLC*

Triglycerides are also split and their reduction indicates an additional fatty acid release of $0.10 \times 3 = 0.30$ mM provided that the triglycerides are completely split in free fatty acids and glycerol The experiments also show that there is a final increase of free fatty acids during incubation of the order of 0 14 mM This net result shows that the remaining part of the fatty acids must have been consumed and probably used in the trans esterification of non esterified cholesterol molecules *The experimental result of a reduction of* free cholesterol of 0 41 mM during incubation gives a certain indication of the correctness of this assumption (Table IV)

Consequently it may be concluded that during incubation a series of lipid transformations take place probably attributable to enzymatic activities In the first place a fatty acid radicle is split off from the lecithin molecule probably the unsaturated one thus forming lysolecithin (lecithinase activity) This fatty acid is transferred to the free cholesterol (fatty acid transferase activity) In addition an ordinary lipase attacking the triglycerides is responsible for further release of fatty acids Some of these will be used for the esterification of free cholesterol which has been shown to decrease to a considerable extent compared with the value before incubation This is

not in accordance with the findings of Glomset et al (13 15) who reported that they had obtained evidence that free fatty acids are not chiefly involved in the plasma cholesterol esterification reaction

Turning back to the original discussion in the introduction to this report the formation of lysolecithin to an extent demonstrated by our experiments certainly results in a diminished sedimentation rate of the red cells after incubation of plasma by transforming them into spherocytes The accumulation of a moderately increased amount of free fatty acids may also act as an additional factor in the disc sphere transformation of the erythrocytes

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INCREASED LECITHINASE ACTIVITY AFTER HEPARIN ADMINISTRATION

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Abstract The increased lecithinase as well as lipase activity of post-heparin plasma in comparison with native plasma subjected to incubation is reported The enzymatic action gives rise to a further increased formation of lysolecithin, a concomitant diminution of lecithin and triglycerides with an appreciable liberation of fatty acids In spite of the presence in abundance of free fatty acids no further esterification of free cholesterol takes place in contrast to the considerable decrease of free cholesterol by transesterification from the enzymatically split lecithin when pre-heparin plasma is subjected to incubation. Possibly an esterification threshold level" exists in this respect. The effect of a single heparin injection on the lipid metabolism will last for about 3–4 h.

Based on the available data from this report and earlier findings the possibility is discussed whether or not the clearing of lipemic plasma can be explained by the consecutive action of a lecithinase and a lipase the first enzyme transferring the stabilizing surface layer of the chylomicra consisting of phospholipids and cholesterol into a more or less water-soluble form, and thereafter the second enzyme can act directly on the chylomicron content of triglycerides. An experimental approach to in vitro clearing of lipemic plasma by the two enzyme systems from extraneous sources is discussed.

Working with the suspension stability of red cells in incubated normal plasma Berlin, 1955 (2) was able to show—in addition to the normally occurring post incubation increase of the suspension stability (Fahræus 1921) (8) i e a decrease of the ESR—that this process was highly augmented if heparin was given intravenously before the blood sample was drawn It was concluded that an increased formation of lysolecithin took place caused by an activation or an increasing release of the normally present lecithinase following the heparin injection

Berlin and Sandring (4) made further experiments in order to obtain more comprehensive information concerning this particular heparin effect They found that very minute amounts of heparin were needed to bring about the increased stabilization reaction far below the doses necessary for the anticoagulant action As small a dose as 500 I U or less could still produce full stabilizing effect and the heparin administration attained this effect almost immediately—after only one minute it was clearly demonstrable and subsided gradually after the lapse of about 3 h The authors also performed experiments showing that an increase of a hemolytically active substance occurred in the incubated plasma samples and this substance exhibited the same solubility properties as lysolecithin (Singer s semi-quantitative method (16)) It was concluded that heparin probably acts as liberator or activator of a lecithinase causing the formation of lysolecithin in addition to its release of a lipoprotein lipase with liberation of free fatty acids Both possibilities were discussed as causative factors for the increased suspension stability of the blood expressed by the ESR changes reported. These findings have been essentially confirmed by the work of Orlando (13) and de Vries (20)

Shore and Shore (15) presented some experimental evidence that heparin may release more than one enzyme and the opinion that lipoprotein lipase is composed of different enzymes has been put forward by Korn (10) and by Zollner (21 22) Vogel and Zieve (18) demonstrated that a conversion of endogenous lecithin to lysolecithin takes place on incubation of plasma for 4 h at 38 C They found no difference in this respect when examining post heparin plasma.

Later Vogel and Zieve (19) have shown by

As demonstrated in Table II there was an obvious increase of the lysolecithin content after incubation of the same order as reported by the present authors earlier (3) amounting to about 0 23 mM and a corresponding decrease of the lecithin level (about 0 21 mM) No difference between native and incubated samples was found as regards sphingomyelin and phosphatidylethanolamine (PE) Free fatty acids and triglycerides were altered in accordance with the findings already reported and the same holds true also for free cholesterol which diminished significantly at least in the female group The number of free cholesterol values in the male group has accidentally been too small to provide a reliable mean

When comparing the results in Tables I and III it has been found that FFA and triglycerides have changed in the ordinary way 20 min after heparin administration On the other hand no obvious change in the level of lysolecithin was found whereas a decrease of lecithin was observed in the male group ($P < 0\ 05$)

After heparin administration essentially the same changes occurred on incubation but to an exaggerated extent (Tables IV and V) Thus an additional formation of lysolecithin was found amounting to about 0 11 mM and simultaneously a further diminution of the lecithin in the order of 0 16 mM The total increase of the lysolecithin above the basic level was thus 0 34 mM and the lecithin decrease was 0 37 mM

There was also an accentuated release of FFA and a decrease of triglycerides in this as in the earlier reported materials There was no significant further increase of the cholesterol esterification after heparin administration

Table III *Plasma lipid and phospholipid values in the same subjects as in Table I after i v heparin but before incubation*

	Women 45–63 y	Men 25–3. y
Free fatty acids, mEq l	0 84 ±0 09	0 88 (0 86–0 89)
Triglycerides, mM	0 6 (0 42–1 08)	0 45 (0 26–0 69)
Total cholesterol mM	6 96±0 67	5 19±0 51
Free cholesterol mM	1 95±0 34	1 26±0 22
Total phospholipids, mM	3 86±0 0	3 10±0 07
Lysolecithin mM	0 1±0 01	0 76±0 01
Sphingomyelin mM	0 85±0 05	0 65±0 03
Lecithin mM	. 75±0 14	2 13±0 07
Phosphatidylethanolamine mM	0 05±0 01	0 05±0 01

Table IV *Changes in plasma lipid and phospholipid values after incubation (6 h 37 C) of the post heparin samples in Table III*

Symbols as in Table II

	Women 45–63 y	Men .5–32 y
Free fatty acids, mEq/l	+1 50±0 23 $P<0\ 001$	+1 09 (0 55–1 62) —
Triglycerides mM	−0 32±0 07 $P<0\ 01$	−0 29+0 05 $P<0\ 001$
Total cholesterol mM	−0 05±0 24 N S	−0 02+0 15 N S
Free cholesterol mM	−0 37±0 12 $P<0\ 05$	−0 4 ±0 18 N S
Total phospholipids mM	−0 07±0 03 $P<0\ 05$	±0 00+0 03 N S
Lysolecithin mM	+0 33±0 01 $P<0\ 001$	+0 35±0 03 $P<0\ 001$
Sphingomyelin mM	−0 01±0 0? N S	±0 00±0 0? N S
Lecithin mM	−0 38±0 03 $P<0\ 001$	−0 36±0 04 $P<0\ 001$
Phosphatidylethanolamine mM	−0 01±0 01 N S	±0 00±0 01 N S

Experiments were also carried out with lipid determinations on different days in the same subject using heparin doses ranging from 2500 I U – 500 I U intravenously The result is shown in Fig 1 indicating that the reported heparin effect could be demonstrated beyond doubt even after

Table V *Differences between the incubation changes (6 h 37°C) of plasma lipid and phospholipid values obtained after and before heparin*

	Women 45–63 y	Men 25–3 y
Free fatty acids, mEq/l	+1 37±0 22 $P<0\ 001$	+0 93 (0 26–1 60) —
Triglycerides, mM	−0 23±0 06 $P<0\ 01$	−0 17±0 05 P 0 05
Total cholesterol mM	−0 17±0 27 N S	−0 06±0 18 N S
Free cholesterol mM	+0 15±0 09 N S	−0 03±0 04 N S
Total phospholipids mM	−0 08±0 05 N S	0 01±0 04 N S
Lysolecithin mM	+0 07±0 01 P 0 001	+0 15±0 03 $P<0\ 01$
Sphingomyelin mM	−0 02±0 02 N S	±0 00±0 03 N S
Lecithin mM	−0 12±0 04 $P<0\ 05$	−0 0 ±0 04 $P<0\ 01$
Phosphatidylethanolamine mM	0 01±0 01 N S	−0 01±0 01 N S

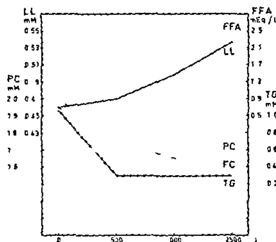


Fig 1 Post incubation phospholipid and lipid values after different doses of heparin intravenously LL = lysolecithin PC = lecithin FFA = free fatty acids TG = triglycerides FC = free cholesterol

1000 I U heparin and probably also—as far as most lipid post heparin changes are concerned—after the minute amount of 500 I U

As regards the duration of the increased post heparin lipid changes the effect lasted at least 3–4 h at which time the lysolecithin level of the incubated post heparin samples approached the level of the incubated pre heparin samples The effect on the triglycerides and FFA lasted even longer

Subcutaneous administration of 5000 I U of heparin resulted in about the maximal response after an initial delay of 1–2 h and the effect subsided after approximately the same time as after intravenously injected heparin

DISCUSSION

From our data it can be concluded that there is present in normal plasma a lecithinase which is able to split the plasma lecithin with formation of lysolecithin and this activity can be further increased by administration of heparin even in minute amounts A simultaneous increase of FFA takes place after heparin administration in addition to the amount already released by incubation of the pre heparin plasma sample These findings may indicate that an exaggeration of the lecithinase activity occurs as an effect of the injected heparin In contrast to the findings of Vogel and Zieve (18) we have been able to demonstrate that there is an essential difference in that respect between pre and post heparin plasma samples An impressive decrease of the triglyceride level takes place with a corresponding release of FFA up to very high levels which is fully in accordance with earlier reports Thus the ordinary lipase activity in native plasma has also in this material been shown largely to increase its effect on the triglycerides in the post heparin samples

The increased formation of lysolecithin after heparin administration which presumably also occurs in vivo might be expected to change the phospholipid pattern obtained in the non incubated post heparin sample A slight decrease of lecithin seems to occur but no change of lysolecithin was found It is probable that the lysolecithin which is formed in vivo is rapidly metabolized e g by the mechanisms described in erythrocytes and leukocytes (7 11 12 14) It may also be interesting in that connection to note that Bergenhem (1) found no increase of suspension stability (shown to be due to lysolecithin formation) when whole blood was incubated during constant agitation As an explanation of this phenomenon he put forward the hypothesis that lysolecithin was restored to lecithin by the erythrocytes

As pointed out earlier by Glomset et al (9) the conversion of free cholesterol to an esterified form takes place by means of a transfer of fatty acid radicles during the enzymatic splitting of the lecithin After heparin injections the degradation of lecithin is increased but remarkably no further transesterification of free cholesterol occurs This is even more unexpected as the concomitant release of fatty acids from the triglycerides offers additional possibilities of esterification of free cholesterol Hypothetically this observation may indicate that this transesterification is in the first place dependent on the presence of lecithin available for immediate molecular transfer of unsaturated fatty acid radicles and not on the presence of fatty acids derived from triglycerides in even abundant amounts The fact that there is no additional decrease of free cholesterol as a result of the heparin administration in spite of a continuing disintegration of lecithin points also to the possibility of a threshold level of the esterification of free cholesterol at least under the present experimental conditions

Thus our report together with the scattered reports of earlier investigators as mentioned earlier in this paper establishes the fact that heparin exerts a definite activity by releasing a lipase and in addition a lecithinase capable of splitting the lecithin molecule into lysolecithin and fatty acids Heparin does not seem to exert any clearly demonstrable effect on the trans esterification between lecithin and cholesterol as no further decrease of free cholesterol was found beyond the level obtained by simple incubation

An important question which arises in this connection is whether the lipoprotein lipase liberated in response to heparin injection is identical with this duplicate enzyme system of lecithinase and lipase As already mentioned it has been suggested that lipoprotein lipase is possibly not a single enzyme but may be composed of two or perhaps more individual enzymes From a theoretical point of view the demonstrated clearing of lipemic plasma by the action of the post heparin clearing factor or lipoprotein lipase can be explained by the consecutive effect of a lecithinase and a lipase on the chylomicra Many investigators have assumed that the chylomicra as well as the other transport form of lipids the lipoproteins are built up of fine aggregates of triglycerides enclosed in a stabilizing surface shell consisting of phospholipids cholesterol and protein There is also some experimental evidence which makes this concept rather acceptable (6)

In our opinion clearing of lipemic plasma may occur in two separate steps at first the lecithinase attacks phospholipids in the surface layer of the chylomicra by splitting the lecithin which initiates the transesterification of free cholesterol also present in the chylomicron shell The lysolecithin thus formed is water soluble In that way the stability of the chylomicron shell may be critically reduced Discussing the problems connected with the stability of chylomicrons Dole and Hamlin found it possible to make a number of general conclusions from the study of artifical emulsions and they emphasize that the combination of water soluble and water insoluble agents such as phospholipids and cholesterol form a more stable dispersion than either agent alone The additive effect of these surfactants suggests in their opinion that they form a mosaic at the surface of the fat droplets

Hence from the experiments of Dole and Hamlin an analogous condition has been thought to prevail in the chylomicron surface layer Therefore when the optimal ratio between the different components of this layer is disturbed it may be broken up and the triglyceride content is open to the direct enzymatic attack of the lipase in a second step of the clearing reaction

Experiments along those lines are at present in progress in order to show whether in vitro mimicry of the clearing reaction is possible to obtain by incubation of lipemic plasma or chyle with different kinds of lecithinase and subsequent introduction in the system of lipase from animal or plant sources

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ACUTE MYOCARDIAL INFARCTION AND PLASMA PHOSPHOLIPID LEVELS

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Abstract. A material of [illegible] cases of myocardial infarction has been analysed with regard to the lipid and phospholipid levels at the acute stage of the disease and at the time of discharge from hospital in 12 cases. Definitely decreased values of lysolecithin have been found which highly significantly differ from a reported normal material. A slight but probably significant decrease of phosphatidylethanolamine has been observed. The FFA level is also considerably elevated in comparison with the normal material. On incubation a lysolecithin increase takes place in the plasma of patients suffering from myocardial infarction as in normal subjects and a concomitant decrease of lecithin occurs. Simultaneously triglycerides and FFA change, the former exhibiting a highly significant decrease and the latter an increase of the same significance level. As in normal subjects an esterification of free cholesterol occurs on incubation. Total cholesterol, total lipid phosphorus and sphingomyelin retain their pre-incubation levels as in the normal material.

The increase of lysolecithin in absolute figures is distinctly below the normal one, indicating that a diminished lecithinase activity has been observed. Cholesterol esterification on incubation is also definitely decreased, suggesting a reduced fatty acid transferase activity.

After the lapse of the initial d[illegible] period the same analyses were performed. At that time most patients had returned to or approached the normal lysolecithin level of their age group. The alterations after incubation at the re-examination are the same as at the initial stage.

The possible biological and clinical significance of the reported findings is discussed.

After the introduction in the late fifties of reliable methods for the determination of individual phospholipids, chiefly by means of thin layer chromatography, these lipid fractions have attracted a renewed interest. Most of the clinical investigational work on this subject has been performed on normal blood and only a few reports have dealt with the phospholipid concentrations in various disease states. Thus Gjone and Mendeloff (6) reported increased levels of lecithin and decreased levels of sphingomyelin as well as lysolecithin in liver diseases of acute and of chronic type, the latter fraction being markedly decreased in cases of biliary cirrhosis. These authors also found a high lysolecithin level in acute pancreatitis. Gjone and Orning (7) recently extended their studies and confirmed the previous reports. They also found that the lowest lysolecithin values were observed in cases exhibiting the most severe liver damage. Petersen (16) and also Phillips (17) had earlier come to essentially the same results. In cases of the nephrotic syndrome Nye and Waterhouse (14) mostly found relatively high levels of the lysolecithin and sphingomyelin fractions, whereas the lecithin level was in general lowered. Hypercholesterolemic subjects have usually shown increased values for all phospholipid fractions, as reported by Vikrot (20). Only scarce reports deal with the phospholipid pattern in atherosclerotic heart disease or in general arteriosclerosis. Nothman and Proger (13) found increased levels of phosphatidylethanolamine (PE) and phosphatidylserine in such patients, however using a colorimetric technique. This finding is not confirmed by the investigations of Wagener et al. (21). As regards the phospholipid concentration in cases of myocardial infarction only a single report has appeared (10), which contained data on phospholipid levels in plasma of a limited material of acute cardiac infarction; a markedly diminished amount of lysolecithin was found in some cases.

The present study will report the results of phospholipid as well as lipid determination in a larger material of acute myocardial infarction and also the same determinations repeated at the time of discharge from hospital in a number of cases.

Table I *Plasma lipid and phospholipid values in patients with myocardial infarction and in healthy controls*

Values given are mean ± standard error of the mean For triglycerides the mean was calculated after conversion to logarithms (4) and the range is shown

n = number of subjects

P refers to the significance of the difference between the two groups N S = not significant

	Acute myocardial infarction	Controls	*P*
Free fatty acids mEq/l	0 86±0 07 *n* = 22	0 52±0 03 *n* = 16	<0 001
Triglycerides m*M*	1 22 (0 57 2 76) *n* = 21	1 07 (0 69–2 22) *n* = 17	N S
Total cholesterol m*M*	6 66±0 31 *n* = 22	6 68±0 43 *n* = 17	N S
Free cholesterol m*M*	1 93±0 11 *n* = 22	2 01±0 21 *n* = 13	N S
Total phospholipids m*M*	3 23±0 11 *n* = 22	3 57±0 16 *n* = 17	N S
Lysolecithin m*M*	0 12±0 01 *n* = 22	0 21±0 01 *n* = 17	<0 001
Sphingomyelin m*M*	0 74±0 03 *n* – 22	0 77±0 04 *n* = 17	N S
Lecithin m*M*	2 33±0 09 *n* – 22	2 55±0 12 *n* = 17	N S
Phosphatidylethanol amine m*M*	0 04±0 003 *n* = 22	0 05±0 005 *n* – 17	<0 05

Table II *Changes in plasma lipid and phospholipid values during incubation (6 h 37 C) in patients with acute myocardial infarction and in healthy controls*

Symbols as in Table I Differences for triglycerides were calculated from the original values

	Acute myocardial infarction	Controls	P
Free fatty acids mEq/l	0 ?6±0 03 *n* – 2.	+0 16±0 03 *n* = 16	0 05
Triglycerides m*M*	–0 10±0 02 *n* = 21	–0 09±0 02 *n* = 17	N S
Total cholesterol m*M*	–0 05±0 08 *n* = 22	–0 07±0 07 *n* – 17	N S
Free cholesterol m*M*	0 26±0 04 *n* – 22	0 45±0 06 *n* 13	<0 01
Total phospholipids m*M*	±0 00±0 01 *n* = 22	±0 00±0 03 *n* – 17	N S
Lysolecithin m*M*	+0 18±0 01 *n* 22	0 24±0 01 *n* = 17	0 01
Sphingomyelin m*M*	+0 03±0 01 *n* = 22	–0 01±0 01 *n* 17	N S
Lecithin m*M*	–0 21±0 07 *n* = 22	–0 24±0 02 *n* = 17	N S
Phosphatidylethanol amine m*M*	±0 00±0 003 *n* = 22	±0 00±0 005 *n* = 17	N S

MATERIAL AND METHODS

Twenty two cases of acute myocardial infarction, 18 men and four women 53–87 years of age have been collected for examination The diagnosis has been established by means of typical case history typical ECG changes serum transaminase elevation temperature reaction and elevation of the ESR Four of the patients died during the initial period of disease three after 2–4 weeks and one after five months The remaining patients are still alive and enjoying relatively good health Most cases were severe or moderately severe one case although typical in every respect was judged to be suffering from a relatively small myocardial damage All patients have been on dicoumarol treatment from the day after admission to hospital but none received heparin

Lipid and phospholipid determinations have been performed before and after incubation as reported in detail elsewhere (1) Fasting blood samples were taken throughout, in the myocardial infarction material as a rule on the morning after admission A control material of 17 normal subjects men and women of about the same age distribution was examined in the same way for comparison with the pathological material In addition 12 myocardial infarction cases were re-examined before the discharge from hospital

RESULTS

Compared with our normal material the present myocardial infarction material shows obvious changes In Table I all mean values for the various lipid and phospholipid values are given as well as standard errors of the mean For comparison our normal values of the same age group are also included in the table In the present material significantly elevated values of free fatty acids (FFA) have been found triglycerides total cholesterol and free cholesterol have shown no marked changes compared with the normal levels As regards the phospholipid pattern there is an obvious decrease of the lysolecithin level during the initial stage after the acute attack of myocardial infarction The lecithin values do not differ conclusively from the normal ones

Sphingomyelin values are completely within the normal range whereas the PE level is probably decreased ($P < 0\ 05$)

As demonstrated before by among others the present authors (1) incubation of the plasma for 6 h at 37 C gives rise to a significant increase of the lysolecithin level and a corresponding diminution of the lecithin level by the action of a normally present lecithinase and concomitantly also a lipase attacking the triglycerides with the release of FFA A direct transesterification of the free

Table III *Plasma lipid and phospholipid values in 11 subjects during the acute stage of myocardial infarction and in the same subjects after about three weeks (in the case of triglycerides ten subjects were investigated)*
Mean ± standard error of the mean is given *P* refers to the significance of the difference N S = not significant

	Initial determination	Repeat determination	Difference	*P*
Free fatty acids mEq/l	0 74±0 09	0 56±0 05	−0 18+0 10	N S
Triglycerides, m*M*	1 28±0 14	1 28±0 09	±0 00±0 12	N S
Total cholesterol m*M*	6 42±0 4?	7 36±0 42	+0 94±0 64	N S
Free cholesterol m*M*	1 81±0 15	2 04±0 12	+0 23±0 16	N S
Total phospholipids m*M*	3 18±0 14	3 18±0 15	±0 00±0.20	N S
Lysolecithin m*M*	0 12±0 01	0 19±0 0?	+0 06±0 01	<0 001
Sphingomyelin m*M*	0 76±0 04	0 79±0 03	+0 04±0 04	N S
Lecithin m*M*	2 26±0 12	2 17±0 14	−0 10±0 18	N S
Phosphatidylethanolamine m*M*	0 04±0 01	0 03±0 01	−0 01±0 01	N S

cholesterol with fatty acids from the enzymatically split lecithin has also been shown to take place (8) Incubation experiments have also been performed in this material and judging from our findings there is obviously a substantial change in the lecithinase and lipase activity As shown in Table II the lysolecithin formation in absolute figures is distinctly below normal as is also the esterification of free cholesterol As the substrates i e the lecithin concentration and the concentration of free cholesterol do not differ from our normal material this finding suggests a diminished enzyme activity

Furthermore a renewed examination of the lipid and phospholipid pattern was made 3–4 weeks after the acute episode usually in connection with the patients discharge from the hospital As shown from Table III the initially decreased levels of lysolecithin have returned to the normal range or showed a marked tendency to do so A single exception in that respect is worth mentioning The patient was admitted to hospital with every sign of acute myocardial infarction with severe pains he was pale and his general condition was poor No unequivocal shock symptoms could be found however Twelve days later his temperature rose from subfebrile levels to almost 39 C enzyme titres increased ECG recordings showed additional changes indicating re infarction The patient recovered gradually but was not afebrile before the beginning of the fourth week in hospital At that time a second phospholipid analysis was made All values had decreased to some degree but most markedly this decrease affected the lysolecithin (0 17–0 08 m*M*) and the PE (0 06–0 04 m*M*)

The results of the incubation experiments at re examination did not differ significantly from the initial values obtained

DISCUSSION

As mentioned before no systematic study seems to have been made regarding the phospholipid pattern in acute myocardial infarction Marinetti et al (10) reported only on a limited material in conjunction with their main investigation of human plasma phospholipids by means of the paper chromatography technique They found decreased values of lysolecithin in a few cases of myocardial infarction but the results are difficult to assess as lysolecithin could not be separated distinctly from inositolphosphatide in their experiments and both compounds were accounted for as a single phospholipid spot even if they attempted to separate the two components by different staining The values obtained were compared with a normal material of only two fasting subjects

In our series of 22 myocardial infarctions only three showed lysolecithin levels just above or at the lowest value of our normal material (range 0 17–0 26 m*M*) all remaining cases had lower values and the mean lysolecithin concentration was significantly decreased ($P<0\ 001$) The significance of the comparatively low values also of the PE has been found to be on the 5% level

In 12 cases we have had the opportunity of repeating the examinations regarding the phospholipid pattern usually just before the patients discharge from hospital In every case but one (the re infarction case which has not been included

in the comparative study) the previously decreased lysolecithin value had returned to or approached the normal range and the three cases showing values around the lower normal border line had all increased to a level well within that range The exceptional case is of special interest as the lysolecithin value had not returned to normal four weeks after the admission to hospital on the contrary a decrease had taken place as an expression of the renewed appearance of myocardial infarction

Our findings from the incubation experiments indicate that certain enzyme activities are reduced The decrease of lysolecithin formation and cholesterol esterification may point to the possibility that the lecithinase activity as well as the fatty acid transferase activity are diminished

The fact that the reported lipid and phospholipid changes after incubation remain essentially the same after the lapse of several weeks may indicate that these reduced enzyme activities are not necessarily connected only with the acute stage of myocardial infarction but may possibly be intimately bound up with the patho-physiological disturbances of the disease

It may be worthwhile to discuss briefly a discrepancy between our infarction material and those earlier reported by others Welin (22) as well as Biorck et al (2) Bjorntorp and Malmrona (3) Tibblin and Cramer (19) and Dodds and Mills (5) have reported a more or less pronounced decrease of serum cholesterol immediately following the onset of a myocardial infarction For some reason we have not found any corresponding change in the cholesterol values

The clinical importance of these observations can so far be merely the subject of speculation One possibility is that the reported phospholipid changes are a biological response to a stress situation of severe pain Against this assumption speaks the fact that the lowest lysolecithin values have not consistently been found in cases exhibiting pronounced shock symtoms or intolerable pains Furthermore no correlation has been found between the lowest lysolecithin values and definitely increased FFA values which would be expected if a secondary catecholamine release had taken place (cf 9) Still the most severe cases have usually been found in the group showing the lowest lysolecithin values

As yet it is impossible to state whether the changes in the phospholipid pattern in acute myocardial infarction is a consequence of the disease or an expression of a biochemical alteration of the milieu intérieur in some way causing the appearance of the coronary occlusion One may speculate as to whether the changes in question may be of importance for the coagulation process per se as the phospholipids (especially the PE) are known to be active in blood coagulation (11 12 15 18) However our data do not warrant any conclusion on this question

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EVALUATION OF THE HYPOTENSIVE EFFECT OF BETA ADRENERGIC BLOCKADE IN HYPERTENSION

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Abstract Thirty-one patients with slight or moderately severe hypertension have been treated with a beta receptor blocker for periods from two to 11 months Twenty patients received the drug as the sole hypotensive treatment, 11 patients were given thiazide before and during the study The treatment resulted in a significant fall in the average mean blood pressure in both groups of patients of an order of magnitude similar to that obtained with thiazide in optimum doses In one patient the treatment had to be discontinued very early because of increasing dyspnoea and gain in weight Apart from this, only minor side-effects were observed More recent investigations seem to indicate that an increase in cardiac output is an essential factor in the pathogenesis of hypertension In this connexion the beta receptor blockers will be particularly important since contrary to other hypotensive agents their effect must be presumed to be related to a reduction in cardiac output

In 1948 Alqvist (1) advanced his theory that the sympathetic nervous system exerts an influence on two different receptors the alpha receptor which is connected mainly with excitatory mechanisms and the beta receptor which primarily exerts an inhibitory function There is one important exception from this rule namely that the purely excitatory effect on the heart of the sympathetic nerves is mediated through beta receptors Of the sympathomimetic drugs noradrenalin stimulates the alpha receptor only adrenalin influences both receptors whilst isoprenaline selectively stimulates the beta receptors Drugs having an alpha receptor blocking ability have been known for a long time whereas the beta receptor blocking substances have been known for the last decade only So far propranolol Inderal® is the beta receptor blocking agent that has been studied most exhaustively

Clinicians have been especially interested in the effect on the heart of the beta blockers This effect is exerted by an inhibition of the formation and conduction of impulses (negative chronotropism) which is utilized therapeutically in the treatment of cardiac arrhythmias In addition the force of contraction of the heart will be restricted (negative inotropism) resulting in reduced cardiac output The effect is particularly pronounced in connexion with exercise restricting the functional capacity of the heart So far treatment of cardiac arrhythmias and angina pectoris is considered to be the principal field of indication Among the less favourable effects exerted by these substances should be mentioned the negatively inotropic action on the heart which aggravates the risk of cardiac failure and broncho-constrictor action which renders these substances inapplicable in patients with asthma

During recent years a few reports have been published relating to treatment with beta receptor blocking agents in hypertension All cases had mild or moderately severe types of hypertension The results seem to indicate that the hypotensive effect corresponds to that obtained when using optimum doses of thiazide The groups of patients studied have been rather small (12, 13, 14 15 17) As regards side-effects a few cases of cardiac failure should be mentioned in particular which were however readily relieved by withdrawal of the drug (14 17) The dosage varied between 80 and 400 mg a day divided into four doses It is pointed out that the treatment did not produce orthostatic hypotension

PERSONAL INVESTIGATIONS

During the past 12 months a number of patients with hypertension have been treated with a beta receptor blocker pharmacologically resembling propranolol, a de

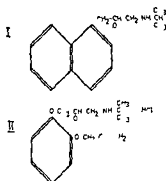


Fig 1 Chemical structures of propranolol (I = 1 isopropylamino 3 (1 naphthyloxy)propane ? 0l)) and Ciba 39089 Ba (II = 1 isopropylamino - hydroxy 3 (0 allyloxy phenoxy) propane hydrochlorid)

rivative of isopropyl minopropane The substance is produced by CIBA and t present is termed 39089 Ba

The effect of this substance according to preliminary studies closely resembles that produced by propranolol An initial dose of 20–40 mg three times a day is recommended maximum dosage 80 mg four times a day

MATERIAL AND METHODS

The study inclu s 31 patients (15 females and 16 males) with mil or m de ately severe hypertension. No special sele tion of the patients was made apart from the fact that the n pertension should not be so severe that temporary withdrawal of previous antihypertensive drugs would be dangerous Furthermore patients with latent or overt cardiac failure or bronchial asthma were excluded from the study

Shortly befo e commen ement of the study all the patients were admitted to hospital for clarification of the aetiology and diagnosis or for a brief check up In none of the patients was any specific cause of the hypertension found The clinical data relating to the patients appear in Table I which shows also the dosage of the beta receptor blocker and the duration of treatment The table is divided into two sections because patients nos 21–31 were treated with thiazide prior to and during the examination (hydrochlorothiazide 75–100 mg a day in a single case polythiazide 4 mg a day) whilst the first 0 patients did not receive other hypotensive drugs

After discharge the patients were followed up on an out patient basis When the level of the blood pressure had been found to be constant on at least three visits to the clini administration of the beta receptor blocker was initiated starting with 20–40 mg three times a day Governed by effects and side-effects the dosage was increased to a maximum of 80 mg four times a day Immediately before and regularly during treatment the following values were determined erythrocyte sedimentation rate haemoglobin, white blood cell count, platelet count, serum concentrations of creatinine chloride bicarbonate potassium alkaline phosphatase and GO-transaminase as well as urinary excretion of albumin and sugar The pulse rate was measured regularly and ECG was checked once during treatment

Blood pressure was determined with the patient in the supine position after 10 min rest and in the upright position after the patient had been standing for two min The determinations were made by a person who did not know about the treatment

The statistical calculations are based on the mean blood pressure MBP (diastolic pressure + $^1/_3$ of the blood pressure amplitude) in the supine position at three determinations before treatment and during the final therapy in each individual patient.

The statistical compilation had a dual purpose First we wanted to know how many patients had experienced a significant fall in blood pressure during treatment ($p < 0.05$) secondly whether treatment had resulted in a significant fall in the average MBP in the two groups of patients

An estimate of the inaccuracy of the blood pressure readings was based on the results found in the 0 patients who did not receive thiazide

The variation of MBP prior to treatment in each individual patient can be expressed as follows

$$s_i = \frac{\Sigma(MBP)_i - MBP_j)}{2}$$

where MBP_i is the average mean blood pressure in each individual patient i indicates each of the 20 patients, and j each of the three determinations of blood pressure carried out in each individual patient

Assuming that the hypothesis $s_1^2 = s_2^2 \quad = s^2$ was valid the best estimate of the variation of the average mean blood pressure before treatment for the entire group of patients was calculated

$$s_{i\,j}^2 = \frac{\Sigma s_i^2}{2\;20} \qquad (1)$$

To ascertain whether the condition mentioned above was fulfilled Bartlett s test (-) for homogeneity between variances was applied The upper 95 limit for $s_{i\,j}^2$ was determined by

$$s_{i\,j_{max}} = \frac{3\; s_{i\,j}^2}{\chi^2_{n=\quad p=0\,05}} \qquad (3)$$

The effect of treatment in the two patient groups was studied by calculating

$$t = \frac{\bar{\Delta}}{s\,\bar{\Delta}} \qquad (4)$$

where $\bar{\Delta}$ is the difference between the average mean blood pressure before and during treatment

RESULTS

In one patient treatment had to be discontinued before any possible change in blood pressure could be assessed (patient no 31 in Table I)

The results of treatment in the remaining 30

Table I *Clinical features daily dose and duration of treatment*

Case no	Sex	Age	Retinal changes (grade)	Heart size (N = normal)	Serum creatinine (mg/100 ml)	Daily dose (mg)	Duration of treatment (mo)
1	o	43	II	N	1.2	320	7
	♀	38	I	N	0 9	160	7
3	o	51	II	N	1 3	3 0	⌞ 5
4	♀	47	II	>N	0 8	320	8
5	♂	39	II	>N	1 5	320	6
6	o	51	I	N	0 9	3⁻0	10
7	♀	64	II	N	1 1	3⌞0	7
8	o	36	II	N	1 0	3 0	7
9	o	38	II	N	1 3	1⁻0	6
10	o	55	II	N	1 5	320	7
11	♀	48	I	N	1 0	3 0	8
12	♀	47	I	N	0 9	3⁻0	8
13	♀	43	I	N	1 4	3 0	9
14	o	59	II	>N	1 2	320	4
15	♂	56	II	>N	1 4	3⌞0	5
16	♀	35	II	N	0 8	160	11
17	♂	47	II	N	1 2	240	5 5
18	♂	48	II	N	1 4	3 0	4
19	♀	39	I	N	1 0	240	5 5
⁻0	o	63	II	N	1 7	⌞00	3 5
Average		47 4			1 18	280	6 6
⁻1	♀	49	II	N	0 9	3 0	7
2⌞	♀	34	III	N	0 8	3 0	⁵
⌞3	♀	50	III	N	2.6	160	5
4	o	58	II	N	1 9	320	6 5
25	o	4⁷	II	N	1 6	60	8
6	♀	45	II	N	0 9	3 0	7
⁻7	♂	41	I	>N	0 8	320	5 5
8	♀	43	II	N	1 0	320	2
9	o	51	II	>N	1 5	3 0	7
30	♀	48	II	N	0 9	3⌞0	11
31	♂	71	II	>N	2.6	160	1
Average		48 4			1 41	267	5 7

patients appear in Table II (the same numbering of patients as in Table I)

The analysis of variance showed as appears from Table III that the variation shown by the MBP in the individual patients was identical in both groups of patients before and during treatment The variation between patients in the non thiazide treated group was significantly greater ($p < 0\,01$) during treatment. Such a change in variation was not observed in the patients treated with thiazide

When applying Bartlett s test, homogeneity was observed between the variances of the average mean blood pressures in the ⌞0 non thiazide treated patients Then $s_{t\ \max(p\ 0\,05)}$ could be calculated and was found to be = ⌞7 mm Hg.

By means of this value six patients can be found in Table II each of whom experienced a significant fall in MBP during treatm nt However the material showed a general tendency to decrease in mean blood pressure during treatment The fall in the average mean blood pressure in the two groups as a whole as appears from Table II is significant ($p < 0\,01$) whereas no significant difference between the results in the two groups is observed

Side-effects

The side-effects are listed in Table IV The only patient in whom treatment had to be discontinued was a 71 year-old man with symptoms of cerebral arteriosclerosis His data are listed in Table I (patient no 31) Before treatment he had a slight tendency to dyspno a on exertion After a few weeks treatment with 120 mg a day the patient developed increasing dyspnoea on exertion and a gain in weight of 2 kg Both symptoms cleared on withdrawal of the beta receptor blocker How

Table II *Mean blood pressures (mm Hg) before and during treatment*

Case no	Mean blood pressure (MBP) Before	Treatment		Average	Mean blood pressure (MBP) During	Treatment		Average	Decrease in MBP
1	137	137	133	136	117	130	123	123	13
2	123	130	128	127	100	100	112	104	23
3	140	142	143	142	135	140	140	138	4
4	130	137	138	135	110	113	123	120	22
5	133	127	1[illegible]7	129	123	113	123	120	9
6	140	140	137	139	103	110	108	107	32
7	143	153	143	146	133	133	127	131	15
8	133	1[illegible]7	137	132	127	117	113	119	13
9	132	133	133	133	107	98	102	102	31
10	133	130	137	133	107	113	113	111	22
11	130	133	137	133	113	113	117	114	19
12	130	123	133	1[illegible]9	123	130	120	124	5
13	130	133	150	138	113	120	123	119	19
14	130	140	130	133	127	140	137	135	−2
15	140	137	145	141	130	127	123	127	14
16	130	117	127	125	113	110	97	103	22
17	123	130	127	127	100	113	107	107	20
18	130	133	130	131	133	137	130	133	−2
19	140	137	125	134	113	103	110	109	25
20	130	133	127	130	108	107	107	107	23
Average				134				117	17
21	132	130	130	131	117	117	107	114	17
22	140	138	133	137	125	135	140	133	4
23	150	150	150	150	107	112	117	112	38
24	143	137	137	139	1[illegible]0	117	123	120	19
25	130	133	140	134	110	103	107	107	27
26	14[illegible]	145	150	146	107	117	107	110	36
27	127	128	130	128	117	122	115	118	10
28	165	173	167	168	143	153	147	148	20
29	137	137	132	135	117	118	127	121	14
30	143	157	153	151	115	115	118	116	35
Average				142				120	2[illegible]

ever the size of the heart had not increased definitely during treatment and no signs of pulmonary congestion were found by auscultation or radiography The patient received treatment in combination with thiazide

Two patients presented slightly increased levels of GO transaminase in serum shortly after commencement of treatment The values became normal within 1–2 months in spite of continued treatment and increased dosage Of course the

Table III *Analysis of variance of the data given in Table II*

Case no	Variation	Stage of the investigation	Degrees of freedom	Sum of squares	Variance estimate	*F* ratio	Significance
1–20 (minus thiazide)	Within patients	Before treatment	2	47	23.5	3.92	None
		During treatment		12	6.0		
	Between patients	Before treatment	19	1705	89.7	4.27	p [illegible] 0.01
		During treatment		7[illegible]78	383.1		
21–30 (plus thiazide)	Within patients	Before treatment	2	30	15.0	2.10	None
		During treatment		62	31.0		
	Between patients	Before treatment	9	4470	496.7	1.11	None
		During treatment		4016	446.2		

Table IV *Side-effects*

Symptoms	No of pats	Dose reduced	Treatment interrupted
Dyspnoea gain in weight	1	0	1
Abnormal transaminase values	2	0	0
Fatigue discomfort	5	2	0

usual pattern of low serum potassium values was observed amongst the thiazide treated patients No changes in this pattern was seen during treatment with the beta receptor blocker and all other laboratory values were constant The ECG showed no changes apart from a slight fall in pulse rate in most patients (on an average from 80 to 73 per min) in no case below 60 per min

Orthostatic hypotension was not observed during the treatment as appears from Table V in which the average systolic and diastolic blood pressure readings at rest and erect are listed

DISCUSSION

The classical concept that the haemodynamic pattern in patients with hypertension is characterized primarily by an increased peripheral resistance has had to be revised during recent years More recent studies indicate that more particularly young hypertensive individuals with a short history of disease and with slight or moderately severe hypertension present normal peripheral resistance but increased cardiac output (3 4 6 10 11) Therefore it is believed that in a number of cases the cause of hypertension is of a central nature such as an increase in the pumping capacity of the heart probably produced by hyperactivity of the sympathetic nervous system and that the increased peripheral resistance occurs later and as a secondary phenomenon—a vascular reaction produced by the increased intravascular tension Indeed in the older age groups and in severe cases the usual haemodynamic pattern is seen

Since it has been proved that the b ta receptor blockers do not reduce peripheral resistance—they might even increase it (3 5 8 9 16)—their hypotensive effect must be exerted through a reduction in cardiac output This is a completely new principle in the treatment of hypertension The results of the few studies so far available confirm the presence of such a mechanism (3 7 16) Therefore it is most likely that in particular young patients with mild hypertension and a short history of disease will be those best suited for treatment with beta receptor blockers In the present study no relationship could be found between the reduction in blood pressure during treatment and the age of the patients the symptomatology the duration of disease or the severity of the hypertension This feature however was not part of the investigation undertaken on the present material

Apart from the case of dyspnoea and increase in weight the drug was well tolerated although the average dosage was high r than that generally given in cases of angina pectoris and cardiac arrhythmias Imminent cardiac failure must be regarded as a definite contra indication As regards the five patients who had some discomfort during the first period of treatment symptoms can hardly be due to any cardiac action of the beta blocking agent as the pulse rate in all these patients was unchanged during treatment and they presented no cardiac symptoms

The significance of the increase in transaminase levels in two patients has not been clarified Since

Table V *Average blood pressure before and during treatment (mm Hg)*

Case no	Before treatment				During treatment			
	Supine		Upright		Supine		Upright	
	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic
1 0 (minus thiazide)	178	112	179	120	156	98	156	106
21 30 (plus thiazide)	187	119	185	1?7	158	101	155	108

it subsided notwithstanding continued treatment this phenomenon is hardly of any major importance It has been described also during treatment with propranolol (16 17)

A definite fall in the average mean blood pressure during treatment was seen in both groups of patients On the other hand with the statistical method employed it was possible to find only six patients whose mean blood pressure showed a significant fall during treatment No doubt a greater number of blood pressure readings would have increased the accuracy of the blood pressure estimation in each individual patient During the day to-day treatment of these patients it is cumbersome to adopt this procedure and therefore it was decided to limit the readings to three

That the variation in MBP between the patients in the non thiazide treated group changed during treatment may be due to the fact that this group comprised a greater number of patients whose MBP did not change essentially during treatment but although the results were apparently better in the thiazide group the difference was not significant.

The investigation showed that an acceptable reduction in blood pressure can be obtained with CIBA 39089 Ba in patients with slight or moderately severe hypertension either with the drug alone or in combination with thiazide On the whole the fall in blood pressure corresponds to hat which might be obtained with thiazide in ptimum doses If the patients are selected according to the criteria mentioned above even better results might be obtained

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MORBIDITY OF PERNICIOUS ANAEMIA

Incidence Prevalence and Treatment in a Danish County

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Abstract On the basis of questionnaires distributed to 124 doctors and eight hospitals in the County of Odense (265 000 inhabitants) Denmark the frequency of per nicious anaemia in the area has been calculated at September 15 1967 There were 334 patients (224 females and 110 males) treated at this date The prevalence was 1 3‰ (1 7‰ in females and 0 8‰ in male) In 60% of the patients the diagnosis was made during a stay in hosp tal and in 70% by examination in a central laboratory or by a specialist

The prevalence is found to be highest in the older age groups, and within these groups there has been a heavy increase over the figures found during a similar survey in 1950 within the same area

The incidence has risen from 8 9 to 9 5 per 100 000 inhabitants since 1950 and assuming the same distribu tion of patients with pernicious anaemia in the entire country as in the County of Odense it can be estimated that e ery year 400–450 new cases of the disease will be d agnosed and that over the whole of Denmark 6000 pa tients are being treated.

Eighty per cent of the patients are treated with hydroxocobemin (Vibeden) and cyanocobalamin tannin complex (Betolvex) Forty per cent of the patients receive more than the recommended dose The sale of vitamin B_{12} preparations within the County in 1966 is however about three times as high as the quantity which is actu ally used for treating the patients with pernicious anaemia and patients with other vitamin B_{12} deficiencies recorded

Pernicious anaemia is frequent in Northern Europe whereas Southern Europeans show a low prevalence rate (3 11) Morbidity studies are however scarce

In 1960 Scott (13) found the prevalence rate of pernicious anaemia in Great Britain to be 1.27‰ whereas Mosbech (8) in 1950 found a prevalence of the disease in Denmark of 1‰

Since it is expected that the rate has increased since earlier surveys because of increasing age of the population and improved diagnostic and therapeutic facilities we have felt it of interest to obtain up to-date figures of the prevalence of pernicious anaemia It was decided to repeat the 1950 survey in the County of Odense Denmark (265 000 inhabitants) Since the sex and age distribution and the distribution by urban and rural districts of the population in this area are representative of the entire Danish population it should be possible on the basis of this study to obtain an estimate of the frequency of pernicious anaemia throughout the country

OWN INVESTIGATIONS

All general practitioners and specialists in internal medi cine and neurology in the County of Odense and all hospitals within the County received questionnaires with the object of collecting data of the patients with perni cious anaemia under treatment at present

Questionnaires were sent to a total of 1 4 doctors and the eight hospitals in the area 123 doctors completed the questionnaires and returned them, one doctor did not wish to partic pate in the study All hospitals gave com plete answers

RESULTS

On September 15 1967 334 patients were on record as being treated for pernicious anaemia in the County of Odense There were 224 females and 110 males Female/male ratio 2 1

The average age of the patients covered by the survey was 70 years Fig 1 shows the age distribution for males and females At the time of study 82 of the patients were over 60 years of age and 54% were over 70

On the basis of the collected data the prevalence rate of pernicious anaemia in the County of

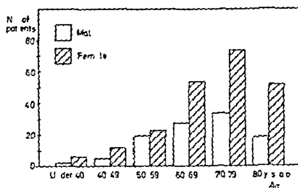


Fig 1 Age distribution at time of investigation

Odense was 1 3‰ (1 7‰ for females and 0 8‰ for males)

Furthermore the prevalence rate of pernicious anaemia has been calculated for various age groups (Table I) and it was found that there is a pronounced increase with increasing age both for females and males

The average age at the time of the diagnosis was 62 years for females and 58 years for males

The incidence of the disease was slightly higher among the urban than among the rural population However no major variations were discovered in particular no accumulation of cases within definitely delimited small areas

The duration of the disease is known in 316 es and the distribution of these cases appears Table II It will be seen that in 70% of these patients the diagnosis was made within the last ten years An average of 25 new cases of pernicious anaemia is diagnosed in the County every year and this rate has been constant for the last ten years On the basis of these figures the incidence is calculated at 9 5 per 100 000 inhabitants per year

Table I *Prevalence of pernicious anaemia in various age groups*

	No of pats		Prevalence ‰	
Age groups	♂	♀	♂	♀
≤ 40	4	6	0 03	0 08
41 50	5	12	0 3	0 7
51-60	[illegible]0	23	1 3	1 4
61-70	28	54	2 5	4 5
71-80	34	74	5 2	10 6
> 80	19	57	9 0	[illegible]4 4

Table II *Duration of pernicious anaemia in years*

	<1	1-5	6-10	11-15	16- 0	>20	Unknown
Males	4	30	37	12	14	7	5
Females	21	80	46	25	18	2[illegible]	13
Total no of cases	25	110	83	37	32	29	18

Attempts were made to assess the criteria on which the diagnosis was based and to what extent the patients had been admitted to hospital in order to have the diagnosis verified Table III shows that the rate of hospitalization is slightly higher among the rural than among the urban population However the figures do not allow any decisive conclusions

In 61% of the patients the diagnosis was established or verified during a stay in hospital Thirty one per cent of the patients had never been admitted to hospital and in 8% of the cases this question was not answered In five of the patients (out of a total of 105) who had not been to hospital sternal puncture had been carried out In 25 of the 105 patients blood tests had been made in a central laboratory or they had been referred for verification of the diagnosis to a specialist in internal medicine or neuro medicine In 65 patients—21%of all patients—the diagnosis was made on clinical symptoms and by means of laboratory investigations which can be carried out in general practice

Data relating to the therapy applied and also on the quantities of drugs administered to registered patients with pernicious anaemia are available for 331 patients Table IV shows the

Table III *Pernicious anaemia place of diagnosis*

	Diagnosed in hospital		Not diagnosed in hospital		Unknown	
	Total	Per cent	Total	Per cent	Total	Per cent
Town of Odense and suburbs	104	62	51	30	13	8
Other urban d stricts	45	52	36	42	5	6
Rural districts	54	68	18	22	8	10

Table IV *Treatment of 331 patients with pernicious anaemia*

Preparation	No of pats	1 ml/ 1 mo	1 ml/ 6 weeks	1 ml/ 2 mo	1 ml/ 3 mo	Unknown		Calculated usage (per y)	Calculated usage according to proposed dosage	Sale in the County of Odense (1966)
Betolvex	81	22	1	28	29	1		570 ml	324– 486 ml	1 193 ml
Vibeden	180	47	27	74	2	10		1483 ml	7 0–1080 ml	3 388 ml
		1 ml/1 week	1 ml/1 week	1 ml/2 weeks	1 ml/3 weeks	1 ml 4 weeks	1 ml 6 weeks			
Cycobemin	38	3	14	16	2	2	1	1578 ml	912–1976 ml	9 010 ml
Exhepa fort	3							165 ml	166– 312 ml	9 0 ml
Hepsol fort	18							1066 ml	468– 936 ml	3 970 ml
Distivit	8							438 mg	292 mg	606 mg
Exopylorin	3							5475 g	5475 g	21 750 g

distribution of the drugs used It appears that *hydroxocobemin (Vibeden) was the agent preferred* since it was used in 55% of the patients

It has been demonstrated (2 5) that complete remission can be obtained by means of hydroxocobemin (Vibeden) and cyanocobalamin tannine complex (Betolvex) and a maintenance dose of 1 mg every second or third month is sufficient The necessary annual consumption of the individual types of drugs can then be calculated as stated in Table IV As regards hydroxocobemin (Vibeden) and cyanocobalamin tannine complex (Betolvex) there seems to be a slight tendency to overtreatment since 40 of the patients receive more than the recommended dose None of the patients *in the group receives less than 1 mg every third* month

In order to obtain data relating to the sale of vitamin B_1 preparations in the County of Odense questionnaires were distributed to the 20 pharmacies serving the area They all completed and returned the questionnaire On this basis the total sale of the individual drugs in 1966 could b calculated

It was reported by the 133 physicians in the area that 30 patients were treated with vitamin B_1 agents because of sequelae following gastrointestinal operations or malabsorption syndromes The annual consumption in this group is 195 ml of Vibeden 24 ml of Betolvex and 62 ml of Cycobemin

It appears from Table IV that the total sale of vitamin B_1 preparations in the County of Odense in 1966 is about three times the quantity used by the 334 patients with pernicious anaemia on re*cord and the 30 patients with B_1 malabsorption* syndromes

DISCUSSION

Adequate lifelong therapy is of decisive importance in the treatment of patients with pernicious anaemia These patients are obliged to se their doctor several times a year for treatm nt or renewal of prescriptions Consequently it can be assumed that the doctors within a specific area will always be aware of the numb r of pati nts with pernicious anaemia under treatment The high percentage of replies and the quality of the answers support this theory

The prevalence rate was calculated at 1 3‰ and as expected it has increased slightly since 1950 at which time a rate of 1‰ was found in a similar study in the same area

The age distribution of the subjects shows a distinct displacement towards older ag groups since the survey in 1950 Eighty two per cent of the patients were over 60 years of age as against 40% previously and 54% of them were over 70 years (formerly 13)

The material presented confirms th assumption that pernicious anaemia is an old age disease with a heavily increasing prevalence rate in the higher age groups These groups show an appreciable increase over the rates found by the survey in 1950 at which time prevalence rates of 3 8 and 3 5‰ were found for males and females respectively in the age group 70–80 years as against rates of 5 2 and 10 6‰ in the present survey In

the age group over 80 the prevalence rates were calculated at 9‰ for males and 24 4‰ for females This increase is more pronounced than would be expected from the prolonged duration of life for the entire population and is presumably due to better diagnostic and therapeutic facilities However a genuine increase in the incidence of the disease cannot be disregarded

Also the incidence has increased from 8 9 to 9 5 per 100 000 inhabitants Assuming a distribution of pernicious anaemia throughout Denmark similar to that found in the County of Odense it can be calculated that 400–450 new cases of the disease will occur annually and that throughout the country approximately 6000 patients will be treated This is actually a 50% increase over the figures found in 1950

In 60% of the patients the diagnosis was made and verified during a stay in hospital This is an actual increase in the frequency of admissions to hospital since the survey in 1950 when only 33% of the patients had been admitted to hospital

The treatment with vitamin B_{12} given as hydroxocobemin (Vibeden) and cyanocobalamin tannine complex (Betolvex) has been adequate in all patients A slight tendency to overtreatment was detected However it is interesting that the sale of vitamin B_{12} in the area concerned is about three times the quantity actually used in treatment of patients with pernicious anaemia and other vitamin B_{12} deficiencies registered This shows that the B_{12} preparations are used rather extensively for other disorders

ACKNOWLEDGEMENT

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THE SIGNIFICANCE OF THE STOMACH TO THE CENTRAL NFRVOUS SYSTEM IN PIGS[1]

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Abstract The gastric fundus is the sole aetiological factor in the degenerative changes of the central nervous system which constantly follow upon total gastrectomy in pigs The cardia and the pylorus are of no importance in this respect

The aetiological relationship between the stomach and the central nervous system was demonstrated by the experiments of Petri et al using total gastrectomy on puppies and pigs (3 4 5 6 7 8 9 10 11 12 13)

To elucidate this finding experiments on pigs were done to study the effect of total operative removal of partly the fundal and partly the pyloric mucosa upon the central nervous system

After primary reports (13 14) the comprehensive experimental material concerning fundal resections has been thoroughly described clinically and morphologically (15 16) and for the CNS system in more detail (4) The investigations have substantiated that the fundus is an important aetiological factor in this respect On the other hand resections of the pylorus had no influence upon the central nervous system (4 13)

Subsequent experiments perform~d by others in an attempt to reproduce the fundal resections on pigs (1 2) seem to have been invariably negative due to certain surgical and diagnostic faults among which was lack of mi roscopy of the central nervous system

PRESENT INVESTIGATIONS

Accordingly the problem is whether the fundus is the primary aet olog cal factor and the other two mucosal areas (cardia and pylorus) of secondary latent significance to the central nervous sys em or whether the fundus is the sole factor

Some of the six, theoretically possible elective resections may contribute to elucidating this question

Four experiments will be reported below

Expe ments 1 and 2 repres nt supplementary reproduction of the previously isolated res ctions of the fundus and pylorus (Fig 1 II in the centre and on the left)

Experiments 3 and 4 comprise partly the lacking isolated resection of the cardia (F g 1 II on the right) and partly simultaneous operative removal of the cardia and pylorus leav ng only the fundus (Fig 1 III in the centre)

The two other combined resections (Fi~ 1 III) need not be considered here as both include removal of the fundus, and the influence of this procedure upon the central nervous system is already known from resections of this region alone

As previously the sequelae of the resections in the nervous system were recorded clinically and examined microscopically The microscopic examination compris d the entire central nervous system including the spinal cord The paradi~m to be used and illustrated in the pres~nt publication however is exclusively the motor anterior horn cells in the cervical spinal cord.

The sequelae of the different types of resections to the other components included in the symptom complex caused by total gastrectomy will not be discussed in this paper

Experiment 1

Elective resection of the f ndus

P g no 16 ♀ age 8 weeks Wei ht and len~th init ally 17 kg 7 cm terminally ~7 kg, 86 cm. Elective resection of the fundus (checked h stotopo~raphically) end to-end anastomosis of the cardia to the pylorus Experimental cond tions as d scribed previously The an mal died spontaneously after an observation period of 5 / months

Cl cal f ndings (terminally)

Nutritional condition greatly affected severe s unting of growth Sk n brownish of a muddy hue hairs long,

[1] Read, in an abbreviated form, by Svend Petri to the Annual Meeting of the Scandinavian Neuro-pathological Associat on January 1968 Aarhus, Denmark.

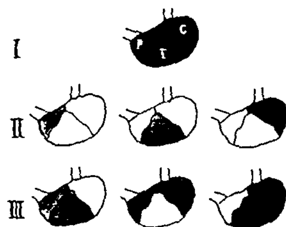


Fig 1 The theoretically possible elective resections of the stomach The dark fields indicate the removed mucosal area or areas I Total gastrectomy II Only one region removed (pylorus fundus or cardia) III Two regions removed simultaneously (pylorus + fundus pylorus + cardia or fundus + cardia)

curled dirty Appetite slightly decreasing faeces normal Blood values initially and terminally Hb 76 67 RBC 5 10–7 3? mill WBC 4? 280– 8 700 reticulocytes 0 7–0%

Signs of very s vere CNS changes like the characteristic sequelae of this type of operation

At autopsy the stomach was found to be of medium size The mucosa represen ed the cardia and pylorus equally without any fundal remnant It was of moderat ckness and normal structure

icroscopi examination showed throughout th cen al nervous system very s vere degenerative changes of the cells and ner e fibres identical in nature and degree with those found in previous investigations with inter alia preponderanc of large pale s vere deg nerations

Result

Removal of th fundus alone resulted in a relatively short tim in the usual very severe chronic CNS d generations



Fig 2 Experiment 1 Very severe clinical signs of CNS changes after resection of the fundus



Fig 3 Experiment 1 Very severe microscopic degenerative changes of the central nervous system. Motor anterior horn cells in the cervical cord—here as in the following figures a paradigm of the findings in the entire CNS

Experiment 2

El ctive resection of the pylorus

Pig no 169 ♀ age 8 weeks Weight and length initially 20 kg 68 cm terminally 89 kg 140 cm Operation elective resection of the pylorus (checked histotopographically) Direct anastomosis of the lower part of the fundus to the duodenum Experimental conditions lik those used previously The pig was killed by exsanguination under general anaesthesia Obs rvation period 11?/ months.

Clinical findings (terminally)

Nutritional state fairly good Moderate stunting of growth Skin and hair normal Appetite and faeces normal Blood values initially and terminally Hb 49 64 % RBC 6 18–9 19 mill WBC 2 217–? ?19 reticulocytes 0–0 2%

No signs of CNS changes Posture and gait as well as entire behaviour normal throughout the observation period



Fig 4 Experiment 2 No clinical signs of CNS changes after resection of the pylorus

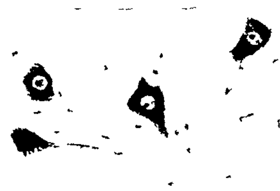


Fig 5 Experiment 2 No microscopic changes in the CNS

At autopsy the stomach was found to be fairly large The greater curvature was 55 cm in length.

The mucosa represented the fundus and cardia equally without any pyloric remnant. It was of medium thickness and normal structure

Microscopic examination of the central nervous system revealed normal conditions in all sites.

Result

Removal of the pylorus alone had no influence upon the CNS system

Experiment 3

Elective resection of the cardia

Pig no 154 ♂ age 7 weeks Weight and length initially 14 kg 63 cm terminally 113 kg 1 0 cm. Operation elective removal of the cardia (checked histotopographically) Direct anastomosis of the oesophagus to the upper part of the fundus Experimental conditions as previously The pig was killed by exsanguination under general anaesthesia Observation period 14 / months

Clinical findings (terminally)

Nutritional state about middling, growth inhibition moderate Skin and hair normal Appetite and faeces normal Blood values, initially and terminally Hb 75–75% RBC 6 9/9 0 mill WBC 16 960–10 800 reticulocytes 0 1–0

For several reasons investigation of the pH in the fasting gastric juice from the experimental and control pigs did not give reliable results

No signs of CNS changes. Posture and gait as well as general behaviour normal throughout the observation period

At autopsy the stomach was found to be rather large Greater curvature 36 5 cm in length Maximum vertical width of the stomach 11 5 cm.

The mucosa represented the fundus and pylorus equally without any remnant of the cardia It was of moderate thickness and of normal structure

Microscopic examination of the central nervous system showed normal appearances in all sites.

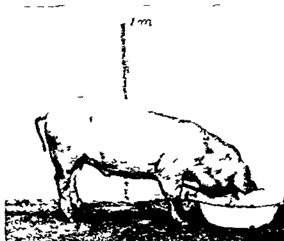


Fig 6 Experiment 3 No clinical signs of CNS changes following resection of the cardia

Result

Removal of the cardia alone had no influence upon the CNS system

Experiment 4

Elective resection of the cardia and pylorus leaving the fundus

Pig no 149 ♂ age 7 weeks Weight and length initially 13 5 kg, 6_ cm terminally 71 kg, 119 cm. Operation elective resection of the cardia and pylorus (checked histotopographically) Direct anastomosis of the oesophagus to the fundus and of the fundus to the duodenum Experimental conditions as previously The pig was killed by exsanguination under general anaesthesia Observation period 13 / months

Clinical findings (terminally)

Nutritional state almost middling, growth inhibition moderate Skin muddy with brownish coating, hair long,



Fig 7 Experiment 3 No microscopic changes in the CNS

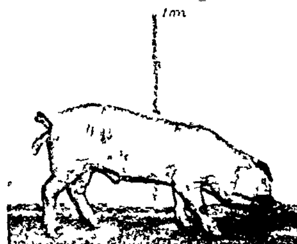


Fig 8 Experiment 4 No clinical signs of CNS changes after simultaneous re ection of the cardia and pylorus leaving the fundus

curled Appetite and faeces normal Blood values initi ally and terminally Hb 60–84° RBC 4 18–8 12 mill WBC 25 280–18 8 0 *reticulocytes 0 2–0*%

No signs of CNS changes Posture and gait as well as general behaviour normal throughout the observation period

At autopsy the stomach was found to be rather large The greater curvature measured 35 cm and the maximum vertical width of the stomach was 15 cm The mucosa represented in accordance with the type of operation exclusively the fundus No microscopic remnants of the cardia or pylorus The mucous membrane which was of its usu l thickness exhibited a normal structure without atr ohy of the epithelium or glands and with out chang f the tunica propria

Micr sc i examination of the central nervous system showe l ı rmal appearances in all sites

Resı

Simult r us operative removal of the cardia and pylorus id not induce degenerative CNS changes



Fig 9 Experiment 4 No microscopic changes in the CNS

RECAPITULATION AND DISCUSSION

Operative removal of the fundus alone (experi ment 1) induced severe chronic degenerative changes of the central nervous system just as in our previous resections of this kind These changes were of the same nature as those found following total gastrectomy

This finding further confirms the primarily decisive aetiological role of the fundal area

Operative removal of the pylorus alone (experi ment 2 and previous experiments) or of the cardia alone (experiment 3) did not affect the central nervous system The same negative result was observed after operative removal of both these regions simultaneously (experiment 4)

In other words the elimination of these two mucosal areas separately or combined did not compromise the ability of the remaining fundal region to maintain the normal status of the central nervous system

In this respect therefore *the fundus is the only gastric factor of aetiological importance*

The absence of influence of the pylorus upon the central nervous system in the pig is remark able considering the significance which the clini cians have attached to this region in assessing the aetiology of pernicious anaemia

In continued experimental investigations into the aetiological relationship between the fundus and the central nervous system previous thera peutic experiments upon an operated pig (and four puppies) must be taken into consideration Vitamin B_6 (permanently administered parenter ally for prophylactic purposes) unlike a number of other *vitamins B studied previously* could *prevent the occurrence of the gastroprival CNS* changes (experimental endogenous avitaminosis B_6) (17 18)

It seems reasonable to relate this specific p e ventive therapeutic effect to the aetiological role of the fundal area

Regardless of the results of further experiments of this nature total resection of the fundus (ex perimental total achlorhydria) in young pigs re presents a method for constant induction of— and studies on—endogenous degenerations of the central nervous system Moreover the investiga tions will presumably acquire clinical and thera peutic importance in human pathology

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A CASE OF TRANSIENT DIABETES MELLITUS IN CONNECTION WITH ACUTE PANCREATITIS

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Abstract A case of diabetes mellitus which developed in a male aged 40 years in association with acute pancreatitis is reported. The patient was admitted with a blood sugar of 10 8 mg % The diabetes required treatment with insulin but the patient recovered completely after 4 / months The literature available is reviewed

Since Harley (15) reported a case of glucosuria in a patient with an abscess of the pancreas in 1862 it has been recognised that disease in this gland can cause diabetic alterations in the carbohydrate metabolism Diabetic keto acidosis in acute pancreatitis was first reported in 1896 by Benda and Stadelmann (15)

The frequency of hyperglycæmia in acute pancreatitis is stated to vary between 10–79 of the cases (2) Glucosuria is stated to occur in from 8–25 of the cases of pancreatitis (4 16 24 25 27)

Hughes (15) collected 18 cases of diabetic keto-acidosis occurring in connection with acute pancreatitis from the literature To these 11 further cases may be added (1 5 7 8 9 10 19 22 26) Out of these 29 patients only six survived and four of them had permanent diabetes Three further cases of hyperosmolar non keto-acidotic coma in patients with acute pancreatitis have been reported (6 10 11) and all of them died

It is difficult to ascertain from the literature how often diabetes mellitus occurs during the course of acute pancreatitis as the majority of accounts do not show whether the diabetes developed during or after the acute pancreatitis Schallenberger (24) found however that diabetes mellitus occurred during pancreatitis in nine out of 45 patients Among Pollock s 100 cases diabetes occurred in six (21)

Only a few cases of prolonged severe diabetic metabolic disturbances have been described in the literature in connection with acute pancreatitis and only two cases have been reported in which the diabetes remitted (10 15) and for this reason the authors considered publication of a further case to be justified

CASE REPORT

The patient was a smallholder now aged 40 years without known predisposition to diabetes mellitus In 1963 and 1964 he had been admitted to the State Mental Hospital in Middelfart on account of endogenic depression. During these periods of hospitalisation the urine was sugar free In 1966 three days prior to admission the patient became tired and very thirsty with a fluid intake of 3–4 l daily Simultaneously polyuria occurred with nausea and occasional vomits but no abdominal pain There were no disturbances of sensation

On account of the above mentioned symptoms the patient was admitted on Dec 17 1966 to hospital elsewhere for diabetes mellitus The blood sugar was 10 8 mg% Forty units of crystalline insulin were administered and the patient was then transferred to Department M The County and City Hospital in Odense

On admission to this hospital the patient was found to be dehydrated He was awake clear and orientated Kussmaul s respiration was not observed The abdomen was normal on admission, but in the course of the next 12 hours it became increasingly meteoristic but without tenderness The temperature was 38 4 C (101 1 F) and it remained raised for nine days

The blood sugar was 8.4 mg% and acetone bodies + + were present in the urine The hæmoglobin was 114% blood urea 116 mg% and standard bicarbonate 27 mEq l

In the course of six hours 1.0 units of crystalline insulin were administered and the blood sugar fell to 1 4 mg% During treatment with parenteral fluids the blood urea and hæmoglobin values returned to normal

Two days after admission the abdomen was still

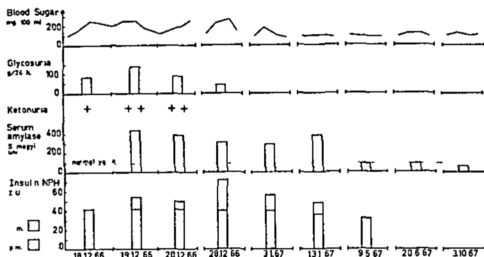


Fig 1 Blood sugar values 24 h excretion of glucose in urine ketonuria serum amylase and treatment with insulin in a male patient aged 40 years with transient diabetes mellitus in connection with acute pancreatitis.

meteoristic and the serum diastase and the urinary diastase were 438 Somogyi units and over 1 00 units respectively

On the basis of these investigations the diagnoses of acute pancreatitis and diabetes mellitus were established

During continued treatment with gastric aspiration, parenteral fluid and Trasylol® the abdomen became normal in the course of four days

The patient was discharged on a strict diet and 3? units NPH insulin in the morning and 1? units in the evening

Investigations

Weight 81–76 kg (180–143 lbs) Height 173 cm (5 7?) ESR 97–10 mm h Serum calcium 8 4–9 8 mg Alkaline phosphatases 8 3 King Armstrong units Serum cholesterol 136–105 mg Triglycerides 1 48 mmol l Fat content in fæces 19 8 g 76 h

The ECG was normal Radiography of the thorax and cholecystography showed normal findings Radiography of the stomach revealed a prepyloric ulcer Ophthalmological examination revealed incipient cataract but no diabetic eye changes

The patient came to out patient examination on Jan 26 1967 and March 30 1967 The blood sugar was well regulated and the patient had not had symptoms of hyperinsulinism During the subsequent month the patient experienced occasional episodes of hyperinsulinism He had no dyspepsia and there was no steatorrhoea He was completely fit for work

During a follow-up admission to hospital from May 8 to 13 1967 the dosage of insulin wa reduced to 20 units insulin retard in the mornings, and insulin treatment could probably have been withdrawn completely It was finally withdrawn during a further period of hospitalisation from June 19 to 27 1967 A glucose tolerance test was undertaken after the patient had received a normal diet for several days and revealed a normal K value (1.38)

The patient was admitted for a final follow up investigation from Oct 2 to 7 1967 As at all the previous follow up investigations the serum diastase was normal The prosecretin test showed normal values and the intravenous glucose tolerance test again showed normal values (K value 1 54)

On examination in June 1967 the patient weighed 67 kg (1?3 lbs) and at the final follow up in Oct 1967 71 1 kg (131 lbs)

Radiography of the abdomen did not reveal any calcification at the site of the pancreas and control examination of the stomach revealed no abnormality

DISCUSSION

The duration of the patient s diabetic metabolic anomaly cannot be determined with certainty but a cautious estimate is approximately 4 ½ months

On the first discharge from hospital the serum amylase had not completely returned to normal and this must be considered to be evidence that the pancreatitis was still active Four months after withdrawal of the diabetic treatment the pancreatic function was completely normal and the intravenous glucose tolerance test showed normal findings

There thus appears to be a close connection between the diabetic metabolic anomaly and the activity of the pancreatic disease

In acute pancreatitis a series of proteolytic enzymes are presumably released and these may have a destructive effect on the pancreatic tissue including the islets of Langerhans (20) It therefore seems reasonable to conclude that diabetes mellitus in these patients may be due to deficiency

of insulin This hypothesis is supported by the investigations undertaken by Rudyi and Chaplinski (23) who found lower insulin like activity in the serum in nine patients with pancreatitis than in a control material Experimental investigations undertaken on dogs by Bridgwater (4) however suggest that glucagon also plays a part in the development of the diabetic disturbance of metabolism in acute pancreatitis

It is somewhat surprising that so few cases of severe hyperglycæmia following acute pancreatitis have been described It is possible that a number of the cases are not recognised because the patients die from diabetic coma without the acute pancreatitis being recognised These conditions have also been emphasized by Holten (14 15) Possibly many cases remain unpublished

Occasional cases of transient diabetes mellitus have been described (12 13 14) None of these cases had had symptoms of acute pancreatitis

The present case emphasizes the significance of treatment not only of the diabetes but also of the pancreatitis

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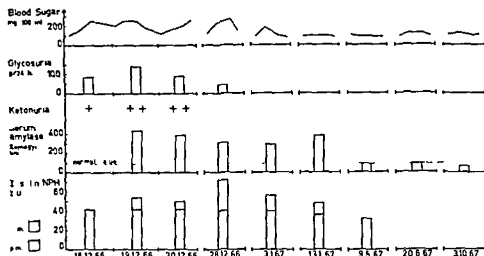


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QUANTITATIVE STUDIES ON THE LIPOLYTIC RESPONSE OF HUMAN SUBCUTANEOUS AND OMENTAL ADIPOSE TISSUE TO NORADRENALINE AND THEOPHYLLINE

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From the Depa tments of Internal Med cine and Su gery Karolinska Hospital and King Gustaf V Research Inst tute Stockholm S eden

Ab tract Subcutaneous and omental human adipose tis sue obta n d at surgery has been incubated in vitro and the releas of glycerol into the medium has been determined

The addition of 0 1 μg of noradrenaline stimulated the ycerol release Maximal st mu ation was obtained with 1 μg of noradrenaline

Theophy ne in a concen ration of 2.5 × 10⁻⁴ M in creas d lipolys s Maximal effect was obtained with 10⁻³ M concentration and higher concentrations tended to inhibit the releas of g ycerol

When maximal stimulation of lipolysis was induced with these two agen s there was a tendency most pro nounced for subcutaneous tissue towards greater lipolys s with theophylline than with noradrenaline

Subcutaneous and omental adipose ti ue had similar basal releas of glycerol The omental tissue however had a significantly higher glycerol re ease than the sub cutaneous tissue when they were maximally s imulated with either noradrenaline or theophyll ne

Free fatty acids (FFA) from adipose tissue supply the body with a great part of its caloric needs This indicates the physiologic importance of this tissue In many diseases the control of mobiliza tion of FFA is disturbed which may cause clini cally significant effects Rat adipose tissue has b en extensively studi d to characterize the re gulation of mobilization of FFA while only a few studies of human adipose tissue are available Although many m tabolic processes are similar in e g rat and human adipose tissue it is becom ing more and more evident that s veral impor tant differences exist between various species especially with regard to regulation of lipolysis (5 7 12) Accordingly we cannot extrapolate from other species to man Basic data have thus to be collected on human adipose tissue

Lipolysis is th process by which the triglyc erides in adipose tissue are hydrolysed to yield FFA and glyc rol The first step in this lipolysis hydrolysis of triglycerides to diglycerides is prob ably the rate limiting step in rat adipose tissue and is mediated by the so-called hormone sensitive lipase (13 14) The activity of this lipase appears to be regulated by the level of cyclic 3 5 AMP (cAMP) (2 4 11) The tissue content of cAMP is controlled by two enzym systems the cyclase which increases and the phosphodiesterase which decreases the amount of cAMP (3 4 9) Studi s on rat adipose tissue have shown that catechol amines stimulat lipolysis presumably by activa tion of th cyclase system (2) On the other hand caffeine and theophylline also stimulate lipolysis but presumably by inactivation of the phos phodiesterase (9 15) While the quantitative aspects of stimulation of lipolysis have b en well documented in the rat epididymal fat pad (1 9 10 17) almost no quantitative studies have been done on the lipolytic dose response of human adipose tissue to catecholamines and theophylline Especially there are no studies in which the dose response to both these compounds has been deter mined

In this paper we have simultaneously studied the lipolytic dos response of human adipose tissue to noradrenaline and theophylline As sub-cutaneous and omental adipose tissue previously were found to behave differently (6) we studied adipose tissue from these two sites

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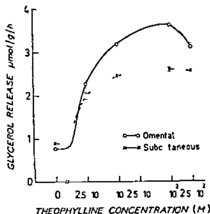


Fig 3 Comparison of the effect of theophylline on the release of glycerol from omental and from subcutaneous adipose tissue from one subject. Each point is the mean of values obtained from five incubation flasks Bars indicate SEM

was obtained in all instances and in both tissues with 1 µg of noradrenaline per ml

Theophylline

Fig 3 shows a dose response curve with theophylline with tissue from one subject The basal release of glycerol from the two tissues was rather similar The release increased about twice at $2\,5 \times 10^{-4}$ *M* theophylline Maximal rate of lipolysis was obtained with 10- *M* concentration

The dose response of tissues from five patients is given in Fig 4 In both subcutaneous and omental tissue maximal stimulation of glycerol release was reached with 10 *M* theophylline There was a tendency for inhibition of lipolysis when the concentration was increased further

Comparison of effects of noradrenaline and theophylline

Table I gives results from studies in ten subjects from whom omental as well as subcutaneous tissue were incubated with both noradrenaline (1 µg/ml) and theophylline (10- *M*) Theophylline caused on the average a greater increase than noradrenaline in the release of glycerol from both subcutaneous and omental tissue This effect however was statistically significant only for subcutaneous adipose tissue Considering the individual values obtained in each patient the following effects of noradrenaline and theophylline were seen In six patients the *subcutaneous* tissue responded with a significantly higher glycerol release after theophylline than after noradrenaline In the remaining four the response of this tissue to the two agents did not differ significantly With regard to the *omental* tissue there was no statistically significant difference between the response to noradrenaline and theophylline in nine of the patients In one however theophylline caused a significantly greater lipolytic response

Comparison of lipolysis in subcutaneous and omental adipose tissue

The basal release of glycerol was similar in subcutaneous and omental tissue (Table I) However omental tissue released more glycerol than subcutaneous tissue when stimulated with noradrenaline as well as with theophylline Looking at individual values nine of ten noradrenaline and seven of ten theophylline studies had significantly higher release from omental tissue than from subcutaneous In the other three theophylline studies there was no statistically significant dif

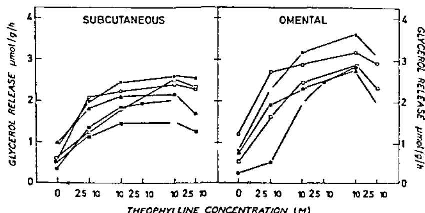


Fig 4 Dose response of omental and subcutaneous adipose tissue from five subjects to theophylline Each point is the mean of values obtained from five incubation flasks.

Table I *Effect of noradrenaline and theophylline on the release of glycerol (μmol/g/h) from subcutaneous and omental adipose tissue from ten patients*

Mean ± SEM

Addition	0	Noradrenaline (1 μg ml)	Theophylline (10^{-3} M)	Theophylline minus noradrenaline	P^a
Subcutaneous adipose tissue	0 61 ± 08	1 09 ± 09	1 57 ± 15	0 49 ± 18	< 0 05
Omental adipose tissue	0 62 + 06	1 94 ± 20	2 23 ± 28	0 29 ± 17	> 0 05
Subcutaneous minus omental	− 0 01 ± 10	− 0 85 ± 21	− 0 65 ± 18		
P^a		< 0 01	< 0 05		

^{a} P value for the statistical significance of the differences

feren e between the tissues In only one patient was the noradrenaline stimulated glycerol release stati tically higher in subcutaneous than in omental tissue

Th studies on the five patients in Fig 2 and the other five in Fig 4 show a similar tenden y with a higher stimulated glycerol release from omental than from subcutaneous adipose tissue

DISCUSSION

As we want d to compare lipo ysis in om ntal and subcutaneous adipose tissue we had to take tissues from anesthetized patients It is not wn if the anesthetics used had any local ef ts in adipose tissue or any general influence g on blood flow or activity of sympath tic nervous system which might have affected the lipolytic process in the tissues However the results on basal lipolysis in this study differ from those we previously observed in human subcutaneous and omental adipose tissue (6) In subcutaneous and omental tissue obtained from 16 patients we previously had an average release of 0 43 ± 0 05 and 0 31 ± 0 03 μmol of glycerol/g/h compared to the present figures from ten patients of 0 61 ± 0 08 and 0 62 ± 0 06 respectively (Table I) In the previous study subcutaneous tissue thus had a higher release of glycerol than omental while these locations had similar basal release in this study The reason for this is not known There are however some differences in these two studies The previous study comprised results from patients who had either received glucose or saline i v in the present the pati nts had only had saline This might have contributed to the difference in results as there was a tendency for glucose infusion to result in higher lipolysis in subcutaneous than in omental tissue (6) Another difference is that Halothane® was not used in the previous study This agent is a good lipid solvent and may thus have an affinity for adipose tissue Halothane® also decreases the capillary resistance in skin and mesentery of cats (18) In general the pati nts in this study were biopsied in a level of anesthesia which was deeper than in the previous study Still another difference between the two studies is that we had here another batch of albumin and in fact the FFA concentration of the present medium was lower than in the former (6) Thus there are several known conditions in these s udies which may have contributed to the dis repancies

Lipolysis has been shown previously to be stimulated by noradrenaline in human adipos tissue (6) No studies are available on the quantitative aspect of this stimulation in human tissu Addition of 0 1 μg of noradrenaline per ml caused a significant increase in glycerol release in most studies and 0 3 μg/ml induced maximal or near maximal stimulation Ten μg/ml of noradrenaline never increased the glycerol release above that seen with 1 μg/ml This indicates that a concentration of 1 μg of noradrenaline per ml is a suitable concentration to obtain maximal respons at least within the ages studied In studies with rat adipose tissue maximal stimulation of lipolysi has b en obtained with amounts of noradrenalin from 0 2 to 2 μg/ml (8 9 17)

Theophylline has previously been shown to

stimulate lipolysis in rat adipose tissue (9 14) Verdy studied the effect of 10 ⁴ M theophylline on the glycerol release from omental adipose tissue of six patients (16) He observed a statistically significant increase in lipolysis in three of these patients We found a significant stimulation of lipolysis in all human adipose tissue samples omental as well as subcutaneous The maximal effect was obtained with 10 M With rat adipose tissue a maximal effect was also seen with 10⁻ M (9) If the effect of theophylline is due to inhibition of the phosphodiesterase this suggests that active formation of cyclic AMP occurs in the tissues without stimulation by addition of e g catecholamines

The maximal stimulation obtained with noradrenaline indicates that the stimulation is a saturable process The tendency observed for theophylline to stimulate lipolysis to a greater extent than noradrenalin indicates the importance of other factors incr asing lipolysis The role of phosphodiesterase in human adipose tissue for regulation of the rate of lipolysis certainly needs further consideration and studies

Higher lipolysis in response to maximal stimulation with either noradrenaline or theophylline was seen in the omental than in subcutaneous tissue This could be due to different amounts of enzym s e g lipase or phosphodiesterase in these tissues It is of importanc to consider differences of this kind in studies when only subcutaneous tissue is biopsied

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Congress Announcements

The VIII International Congress of Nutrition will be held in Prague August 28 to September 5 1969

Organizers International Union of Nutritional Sciences (IUNS) and J E. Purkyně Czechoslovak Medical Society Section for Gastroenterology and Nutrition

President J Mašek D Sc M D

Secretary General Z. Slabochová M D

Office of the President and the Secretary General Institute of Human Nutrition Budějovická 800 Prague 4

Congress languages English French German Russian

The II International Congress of Psychosomatic Medicine organized by the French Society of Psychosomatic Medicine will be held in Paris Septemb r 17 to 20 1970 on the theme The Psychotherapeutic Action of the Physician

Information from S O C F I 7 rue Michel Ange Paris 16[e] France

Acta med scand Vol 185 pp 471–477 1969

HEART VOLUME AND ITS RELATION TO MEASURES OF CIRCULATORY FUNCTION IN HEALTHY YOUNG MEN

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Abstract Several roentgenological methods for determination of the heart volume have been presented. In order to evaluate the influence of different projections and body positions on two of these methods the relationship between the heart volume and different measures of the circulatory function capacity was studied in a series of healthy 20-year-old subjects In the heart volume determination seven different projections and positions were used and two formulae were applied For each of the 14 methods of calculation, the regression line and residual variance were calculated as well as the coefficients of correlation for the different function parameters Special attention was paid to the relationship between the work load at a pulse rate of 170 per min (W_{170}) and the heart volume The regression equations obtained deviated considerably from those described previously A high correlation was found however between the heart volume and (*a*) W_{170} and (*b*) the oxygen pulse among other parameters.

Roentgenological measurement of the volume of the heart is a common investigation which is often of great importance in the evaluation of cardiac diseases Thus an accurate and precise method is obviously of value In normal persons the heart volume is related to the size of the body and the circulatory function capacity inter alia and normal limits must be set in relation to one or more such parameters

Both information from the literature (9) and our own experiences from heart volume determinations on both human beings and models (3 4 6) have provided new viewpoints concerning previously described relationships between heart volume and physical work capacity (11) A fundamental factor in the discussion of these questions is the validity and reproducibility of the roentgenological method This method is not as yet fully satisfactory We have therefore attempted to find the best roentgenological method for heart volume determination with the use of the formulae of Jonsell (13) and Kjellberg et al (15) by studying in a limited series of subjects certain relationships between anthropometric data and circulatory parameters The aim was not to demonstrate significant relationships since in many cases these have already been demonstrated previously but to find the best degree of accuracy in prediction of the heart volume in the individual case from parameters for the circulatory function capacity

This investigation forms part of a methodological study of the heart volume in different age groups for both sexes which is in progress

MATERIAL

The investigation was carried out on a series of 20 subjectively and clinically healthy men of ages 19–25 years The majority of the subjects were medical students, and the remainder hospital personnel. None of them were élite athletes, but their degree of physical training varied considerably (intentional selection) The body height of the subjects ranged from 172 to 191 cm, the body weight from 60.5 to 83 kg and the body surface area from 1.71 to 2.10 m² (determined from nomograms from height and weight data)

PHYSIOLOGICAL METHODS

The physical work capacity was determined by bicycle ergometry (19) with graded load of 300–600–900 etc. kpm/min Each load stage comprised 6 min. By interpolation or extrapolation the work intensity at a heart rate of 170 per min (W_{170}) could be determined (submaximal test) assuming a linear relationship between the work loads and the pulse rate after 6 min at each load When the heart rate had increased to about 170 per min the work load was increased by 100 kpm/min for each minute until the subject discontinued the test because of

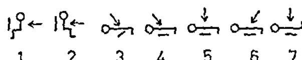


Fig. 1. The seven different projections and positions are shown by the symbols above.

exhaustion (maximal test [ill] (7)). During the 6-min loads expiration gas was collected in a Douglas bag for determination of O_2 consumption and CO_2 elimination. Gas analyses were performed according to the method of Haldane with the apparatus modified by Engholf. From O_2 consumption values the oxygen pulse (oxygen uptake/heart beat) was calculated at the load of 900 kpm/min. During the final part of the exercise test (the one minute loads) heart rate determination alone was performed. At the end of the test and 3 min after its discontinuation capillary blood was taken from a finger tip for blood lactic acid determination. These analyses were performed according to Barker and Summerson (2) with a slight modification omitting the initial copper-calcium treatment. Before the start of the test recordings were made at rest and also after 8 min of passive standing, of the blood pressure, heart rate and ECG. ECG recordings were also made during and after exercise.

On a day near to the day of the exercise test plasma volume (PV) determination was performed by the Evans blue dye method in detail according to Wiklander (21). The PV test was performed in the morning with the subject in a fasting condition after 30 min rest in the supine position. In addition the hematocrit (Hct) and the hemoglobin (Hb) concentration in venous blood were determined. From the values for PV, Hb and Hct the total Hb (THb) was calculated assuming the body Hct to be 0.90 of the venous Hct.

In order to characterize the series of subjects a number of anthropometric measurements, in addition to body height and body weight, were made, among others the biacromial breadth. Further, certain lung function tests were carried out (vital capacity and maximal ventilation determinations) and these showed, as did the ECG recordings, normal conditions.

ROENTGENOLOGICAL METHODS

All patients were examined in the prone and supine positions and also in the sitting position both with the front of the chest and with the back against the film. The frontal projections and positions used can be seen in Fig. 1. In the lateral projections the beam direction was always at right angles to the sagittal plane of the patient. Projection 1 was described by Jonsell (13), projection 4 by Kjellberg et al. (14) and projection 5 by Larsson and Kjellberg (17). The film focus distance was 1.5 cm. The frontal and lateral films were exposed simultaneously and the exposure was triggered by the *R* wave in the simultaneously recorded ECG. The apparatus used for this was an ECG trigger manufactured by Elema Schönander AB, Solna, Sweden. The delay time varied between 0 and 1.0 sec. In each individual subject the exposure in the different projections and positions was made during the same period in the cardiac cycle. Thus 12 subjects were examined in systole and 8 in diastole.

Measurements on the films were performed by two of the authors in collaboration (K. B. and U. E.) and in all investigations the volumes were calculated according both to the method (here called "J") described by Jonsell (13) and to the method (called "K") described by Larsson and Kjellberg (17) and Kjellberg et al. (14, 15). In these calculations it was taken into account that the *J* equation is intended for frontal projection at right angles to the frontal plane of the patient and the K equation for frontal projection at an angle of 30°. When the *J* equation is used for angled frontal projections, and the K equation for right angled frontal projections, a correction factor therefore has to be introduced. The equations are given below. At the projections which deviate from the original projection of the respective authors the corresponding correction factor is given. Fig. 2 shows how the measurements of the diameters were made.

Volume (*V*) according to (*J*)

$$V = \frac{\pi}{6} a \cdot b \cdot c \cdot \frac{(f-v^f)^2 \cdot (f-v^l)}{f^3} \quad (1)$$

where *a*, *b* and *c* are the diameters given in Fig. 2, *f* is the film focus distance (= 175 cm) and v^f is the distance from the centre of the heart to the frontal film and v^l the same distance to the lateral film; this makes it possible to evaluate the magnification.

Volume (*V*) according to (*K*)

$$V = \frac{\pi}{6} \sqrt{1 - \frac{c_1^2}{3e_1^2}} \cdot d \cdot e \cdot c \cdot \frac{(f-v^f)^2 \cdot (f-v^l)}{f^3} \quad (2)$$

where c_1 and e_1 are *c* and *e* reduced for the magnification. The other symbols *f*, v^f and v^l are as in the *J* equation, *d*, *e* and *c* are as in Fig. 2.

When equation 2 was used for right angled frontal projections, i.e. 1, 2, 3, 5 and 7 in Fig. 1, the volume was corrected by $2/\sqrt{3}$ and when equation 1 was used in projections 4 and 6 it was corrected by $\sqrt{3}/2$.

The volumes were calculated by a computer.

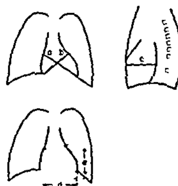


Fig. 2. The three diameters according to Jonsell (*a*, *b* and *c*) and the three diameters according to Kjellberg et al. (*d*, *e* and *c*) are shown.

Table I *Heart volume in ml The mean valu s ($\bar{X}$) standard deviation of the mean (S) and range for the 20 subjects are given for each projection and for the two different formulae*

The numerals 1-7 refer to the different projections and po itions (see Fig 1) *J* means that the formula of Jonsell was used and *K* that of Kjellberg et al

		1	2	3	4	5	6	7
J	$\bar{X}$	684	668	678	708	754	692	776
	S^-	27	21	17	19	17	0	20
	Range	410–850	430–791	533 803	557–950	617–869	539–852	64 –921
K	$\bar{X}$	686	679	678	731	767	730	760
	$S_{\bar{x}}$	28	23	20	17	19	22	20
	Range	425–888	458–913	5?0–842	603 877	648–966	561–870	612–936

RESULTS

A *Heart volumes*

The different volumes in the seven projections and positions are shown in Table I where the mean values the standard deviation of the mean and the range are given In the individual subjects the volume variations between the different pro jections were relatively large in the recumbent positions This is treated in greater detail in an other paper (6)

B *Physiological parameters*

The most important physiological and some an thropometric data are given in Table II where the mean values $\bar{X}$ the standard deviation of the m an $S_{\bar{x}}$ and the range are shown

C *Relationship between heart volume and physiological parameters*

We have chosen to report primarily the relation ship between the heart volume obtained with the different projections and positions described above (Fig 1) and the physical work capacity expressed as W_{170} i e the work load at a heart rate of 170/min during leg exercise on an ergometer bi cycle Figs 3–5 show the different relationships with regression lines and S (S^2 = residual vari ance) given for each of three positions and pro jections Corresponding regression equations and correlation coefficients r are given in Table III Table IV show the relationship between heart volume and body surface area

Table II *The mean values ($\bar{X}$) standard deviation of the mean (S) and range for physiological and an thropometric data*

	$\bar{X}$	S^-	Range
W_{170} kpm/min	1107 5	38 0	825 1350
Body surface area m^2	1 91	0 03	1 71 2 10
O_2 uptake ml/m n at load of 900 kpm/min	2243 4	23 3	-030 2457
Oxygen pulse at load of 900 kpm/min	14 85	0 35	12 17
Heart rate rest	70 1	3 0	48 100
Heart rate after stand ng 8	83 1	3 61	55 120
Max heart rate during exercise	19? 9	1 18	180– 00
Max blood lactic acid mg	98 6	5 60	47 0–141 0
Hb g/100 ml	14 9	0 25	12 7 16 8
Rad o ulnar breadth cm	6 0	0 06	5 5 6 3
Femoro-condylar breadth cm	9 8	0 08	9 1–10 5
THb g	837 1	42 4	468–1155

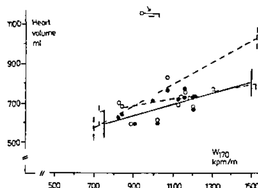


Fig 3 The heart volumes in the prone position in ml (ord nate = y) in relat on to physical work capacity W_{170} in kpm/min (abscissa = x) Open circles (O) represent volumes calculated according to the *K* formula and filled circles (●) volumes calculated according to the *J* formula

Three regression lines are given (*a*) for volumes cal culated accordin to Jonsell (——) (*b*) for volumes cal culated accordin to Kjellberg et al (– – –) and (*c*) the line found by Holmgren et al (– – –) The regression equations for ( ) and (*b*) are listed in Table III The re gression equation found by Holmgren et al was $y = 0\,558\,x + 190$ $S = 93\,3$ ml $r = 0\,93$ Here S means (residual variance)$^{\frac{1}{2}}$ ($\pm 1S$ is given in the figure for each regres s on l ne) r is the coefficient of correlation According to Holmgren and Strandell (1959) the equation should be $y = 0\,588\,x + 190$

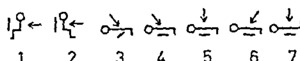


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Volume (*V*) according to (*K*)

$$V = \frac{\pi}{6}\sqrt{1 - \frac{c_1^2}{3e_1^2}}\; d\, e\, c\, \frac{(f - v^f)^2\,(f - v^l)}{f^3} \tag{2}$$

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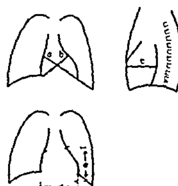


Fig. 2. The three diameters according to Jonsell (*a*, *b* and *c*) and the three diameters according to Kjellberg et al. (*d*, *e* and *c*) are shown.

Table V *The correlation coefficients on using the heart volume formulae of Jonsell (J) and Kjellberg et al (K) showing the correlation between volumes calculated for different projections and different physiological parameters*

The numerals 1–7 refer to the projections shown in Fig 1

Projection	1	2	3	4	5	6	7
Oxygen pulse (*J*) at 900 kpm/min	0 66	0 64	0 55	0 59	0 58	0 41	0 54
Oxygen pulse (*K*) at 900 kpm/min	0 58	0 72	0 45	0 38	0 48	0 52	0 56
Radio ulnar breadth (*J*)	0 32	0 [illegible]4	0 33	0 49	0 41	0 51	0 28
Radio-ulnar breadth (*K*)	0 17	0 26	0 34	0 39	0 52	0 56	0 35
THb (*J*)	0 45	0 30	0 51	0 36	0 43	0 44	0 60
THb (*K*)	0 40	0 48	0 38	0 34	0 50	0 32	0 63
Max blood lactic acid (*J*)	−0 08	0 08	−0 2[illegible]	−0 10	−0 23	−0 [illegible]2	−0 15
Max blood lactic acid (*K*)	−0 20	0 06	−0 25	−0 30	−0 25	−0 03	−0 19

gren et al (11) and Holmgren and Strandell (12) who reported the best linear relationships between on the one hand heart volumes (determined according to the K method (14 15 17)) and on the other physical work capacity and total hemoglobin Nor did we find so high a correlation coefficient between these physiological parameters and the heart volume as was reported by Kjellberg et al (14 16) This deviation between previous investigations and that presented here may be due to differences in 1) the physiological method 2) the roentgenological method and/or 3) the material

Ad 1 The physiological method appears to be valid The physical work capacity was determined by the method currently used in Sweden Hellstrom and Holmgren (10) in a series of young healthy conscripts who were investigated with double determination of W_{170} found good agreement between two tests They calculated the standard error at a single determination of W_{170} and found it to be 55 kpm/min (or 4 9 % of the mean) External environmental conditions may play a part in estimations of the work intensity from the heart rate but the room temperature which may be an important factor in this respect did not vary in the laboratory to such an extent that it could have influenced the results noteworthily The differences from previously reported regression equations were probably not due to a lower precision or reliability in our determination of W_{170} than in the earlier investigations Calculations of work capacity based on oxygen uptake values are however more reliable For certain projections (Table V) a relatively high correlation coefficient for the oxygen pulse was obtained The relationship between oxygen pulse and heart volume has also been studied in earlier investigations In a number of studies from Germany (cf 18) the maximal oxygen pulse was used as a circulation parameter and good correlation was found between this and the heart volume Oxygen uptake determinations during a work test cannot be used as a clinical routine method however in all cases in which a heart volume determination is indicated which limits the practical value of such a correlation

In clinical practice the heart volume is usually related to the body surface area This correlation may be expected to be best in persons with a small amount of adipose tissue as in the case of the present series of subjects It is evident however both from the present series and from previous reports (14) that a somewhat lower correlation is obtained between the heart volume and the body surface area than between the heart volume and the best measure of circulatory function capacity for the majority of the projections used and with both methods of calculation (Tables III and IV)

Ad 2 In the heart volume determination difficulties were sometimes encountered in determining the end points of the diameters according to the *J* method or in deciding how the upper and lower tangent should be drawn according to the *K* method This applies accordingly to the lower tangent also in a number of cases when using the *K* projection (projection 4 Fig 1) This is a conceivable reason for deviation from the true

heart volume and the relative error may vary from one case to another. The volume studies on heart models carried out by Bergstrom and Eriksson (3) and Bergstrom et al. (4) showed that even in volume determinations on models considerable differences may be obtained between the calculated and the true volume, in spite of the fact that the contour of a heart model in its entirety is well delimited and defined. Thus the mathematical approximation constitutes a further cause of deviation. To judge from double determinations the random error of a single determination by the A method may be small—at the best 3–4 per cent. Hellstrom and Holmgren (10) thus found in their series that the standard error of a single determination at rest was 4.2 per cent of the mean and Ekelund et al. (8) found the coefficient of variation during exercise in the supine position to be 4.2 per cent.

The exposures were triggered by the *R* wave in the ECG. The time delay between the *R* wave and the exposure was set to the shortest possible allowed by the apparatus, but nevertheless varied considerably between individuals. The different exposures in one and the same individual took place, however, in the same phase of the cardiac cycle. The variations between the different individuals gave rise to yet another possibility of uncertainty in the volume determination which was thus made either in systole or diastole. We therefore carried out a separate study of 20 who had undergone biplane angiocardio aphy in the supine position with an exposure ency of six films per second and simultaneous ECG recording (5). This study showed that there was no definite difference in total volume between systole and diastole. Thus the point of time for exposure within the cardiac cycle should have had no influence on our results. A similar conclusion was drawn by Kjellberg et al. (15), also valid for the horizontal body position but by a different method.

Our study showed that the A method of investigation with an angled frontal beam (projection 4) did not give more reliable results than the simpler method with the patient in the supine position (projection 7) and with a right angled frontal beam (see Tables III and IV). The results emphasize the value of further standardization of the roentgenological methods of heart volume determination and the necessity of continued studies of both the formulae and the methods of measurement. We assume that such studies of the formulae may lead in the future to new equations and increased reliability.

Ad 3. Previous investigations in which the relationship between the heart volume and W_{170} has been studied have been based on series of varying composition of subjects. Large series of subjects have as a rule had a wide age range, while those series which have consisted of uniform age groups have often been small, even smaller than our own. The only large series with a narrow age range (conscripts and others) was presented by Hellstrom (9). He made the same observation as we did, namely that for persons with a relatively high W_{170} a smaller heart volume was often obtained than was expected from series with a wide age range. For large numbers of his subjects the conventional regression equation which has been calculated from an inhomogeneous series as regards age and sex (11) could not be used for predicting W_{170} from heart volume values. Other kinds of discrepancies have also been described. In another series of conscripts of three different groups Ahlborg (1) found that between two of the groups there was a significant difference in the heart volume but no significant difference in W_{170}.

In a subject material which is uniform as regards age it is conceivable that individual differences in degree of physical training and peripheral oxygen utilization may play a part in the relationship between heart volume and physical work capacity. The observations of Hellstrom (9) support this possibility. He found different regression equations for the relationship between heart volume and W_{170} for different types of athletes (weight lifters and runners) and also slightly altered equations for one and the same group (students at the High School of Gymnastics and Athletics, Stockholm) at investigations on different occasions. In addition to training variations there are certainly age variations. Strandell (20) found that men of high ages showed a significantly larger heart volume per THb than younger men. From their large series of subjects Reindell et al. (18) also found different regression equations for the relationship of maximal oxygen pulse to heart volume between men and women, between different ages, and between male athletes and men of ordinary physical training.

It is evident from the above that the relationship

between heart volume and W_{130} need not be linear or identical for different age groups Our series is too small for a comprehensive regression analysis but there seems to be good reason in further studies of this relationship to take into account differences in age and sex and degree of physical training among other factors

Appendix. *Statistical methods*

The following statistics were used

n number of observations

x_i y_i the ith observation of the variables X Y

1 Mean

$$\bar{X} = \frac{\sum x_i}{n}$$

2 Standard deviation

$$S = \left[\frac{\sum x_i^2 - n\bar{X}^2}{n-1}\right]^{\frac{1}{2}}$$

3 Standard deviation of the mean

$$S_{\bar{x}} = \frac{S}{\sqrt{n}}$$

4 Correlation coefficient

$$r = b\left[\frac{\sum x_i^2 - (\sum x_i)^2/n}{\sum y_i^2 - (\sum y_i)^2/n}\right]^{\frac{1}{2}}$$

where

$$b = \frac{n\sum x_i y_i - \sum x_i \sum y_i}{n\sum x_i^2 - (\sum x_i)^2}$$

5 Regression line $y = bx + a$

b see above

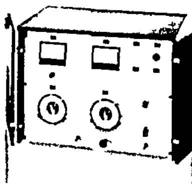
$$a = \frac{\sum y_i - b\sum x_i}{n}$$

6 Residual variance

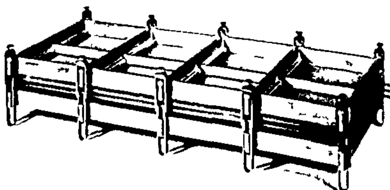
$$S^2 = \frac{n}{n-2} S_y^2(1 - r^2)$$

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AUTOMATIC CONTROL UNIT



LOW RESISTANCE DIALYZER UNIT

Acta med scand Vol 185 pp 479–481 1969

ORAL CONTRACEPTIVES AND CEREBRAL ARTERIAL THROMBOSIS

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Abstract Attention is called to the causal relationship between the occurrence of cerebral arterial thrombosis and the use of oral contraceptives in several instances Two such cases are briefly described, the ages of the patients being 40 years in one case 17 years in the other Necropsy confirmation available

Conclusive evidence has been presented for a correlation between the use of oral contraceptives and the occurrence of cerebral thrombosis (2 4 6 7 12 18 19) Two similar instances will be reported here

Case 1

Woman, aged 40 mother of three Mother and six out of 1[illegible] sibs are said to have had toxic goiter She has used oral contraceptives for the last three years. During this period her blood pressure has been slightly increased. Almost two years before admission to the clinic she was operated upon for herniation of a lumbar disc with reduced sensibility of her right leg as a consequence She worked as a secretary in our hospital Two weeks prior to admission she had an attack of headaches, with temporary disappearance of visual acuity of the right eye She has had this phenomenon repeatedly in the last few months.

She was admitted to our clinic on March 13 in the early morning about 5 o clock her husband had noticed some motor activity in his wife her speech was slurred, she said she had acute headaches and she could not move her left arm or left leg.

On admission partly conscious, left-sided hemiparesis, blood pressure 155 100 pulse rate 100 extensor plantar response of her left foot. Arteriography of the carotid arteries revealed nothing conclusive and she was prepared for a vertebral arteriography However she got worse relapsed into coma a rigidity of all four extremities, more so on the left side was noted Babinski positive on both sides. She died on March 22, 1968

Autopsy showed fresh thrombosis of 20 mm of the intracranial part of art. vertebralis dx., the whole art. basilaris and the proximal 5 mm of art. cerebelli inf post dx. and mm of art. cerebri post dx. (Figs. 1 and 2) with infarction of the right cerebellar hemisphere and parts of the left cerebellar hemisphere and the pons. There were signs of increased intracranial pressure with cerebral edema and a small infarction of part of lobus occipitalis sin. The art. vertebralis sin. was considerably narrower than normal but without thrombosis. No atheromatosis could be detected by the naked eye in the intracranial arteries. There was a slight atheromatosis in the aorta, coronary arteries, renal arteries and other large arteries. The heart was of normal size with no signs of hypertension.

Microscopical examination showed a very slightly thickened intima within a restricted part of circulus Willisi.

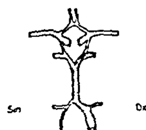
Case

Woman, aged 17 Admitted to our Neurosurgical Department (Professor Bohm, M.D.) on Apr 10 1968 Use of oral contraceptives since Oct. 1966 Hampered with headaches since this time has used aspirin in considerable amounts. Apr 4 1968 acute epileptiform seizure with incontinence of urine She was taken to her rural hospital presenting on arrival left-sided hemiplegia, deviation conjugée towards her right side Babinski positive left foot. Eyegrounds normal blood pressure 145 105 heart rate 1[illegible]0 She was brought to our Department of Neurosurgery on Apr 10 unconsciousness, epileptiform seizures, pupils rigid to light. Angiography The right carotid artery tapers off from the siphon, whereas the external carotid was adequately filled. She went rapidly downhill and died on Apr 11

Autopsy showed fresh thrombosis of the intracranial part of art. carotis int. dx. (Fig. 3) with infarction of lobus frontalis dx. and the rostral part of lobus parietalis dx., several small pontine hemorrhages, and signs of increased intracranial pressure There was no atheromatosis in the intracranial arteries. There was an abundant pulmonary edema. No other findings of interest were made Microscopical examination showed a normal arterial wall at the site of the thrombus. Heart weight 250 g.

COMMENTS

The first case a woman aged 40 had partaken of oral contraceptives for years because of increased bleeding during her periods Although only a very discreet atheromatosis was to be noticed a[illegible] the necropsy it must not be forgotten that the plasma lipids may become increased [illegible] the use of these drugs (8 13 17 20) The



Fi 1 Diagram of the extension of the arterial thrombosis in case 1 The thrombosis obviously had its origin in the right ertebral artery and the pos erior part of th ba i ar ar ery

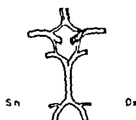


Fig 3 Diagram of the extension of the arterial thrombosis in case 2

tion to the right vertebral artery is of some in er e t In 11 out of 12 ordinary ins ances of Wallenberg s syndrome th left vertebral artery is involved This is probably so b cause of hydrodynamic differences b tween the two ver ebral arteri s the right one leaving the subclavian artery in a recurrent direction However in this cas in th fatal case de rib d by Ethisamuddin (11) and in three other observations at my (A U) disposal all of which had partaken of oral contra c ptives the right vertebral artery was the one involved

The second case a previously healthy young woman had used oral contraceptives for about 18 months It is of some interest that headaches appeared during this period which is a rather common phenomenon wh n partaking of those drugs (3 4 15) She died six days after the



Fig The thrombosis in case 1 1 ca sed an infarction of most of the cerebe um, particu arly its right half

onset of her disease a thrombosis of the right internal carotid in its intracranial part Incidentally this localization is far from uncommon

In our Department of Pathology the material of pulmonary embolism cerebral arterial thrombosis and hemorrhagia cerebri was scrutinized for the period January 1 1962 – April 30 1968 The total number of necropsies during this period was 6158 that is about 1000 a year with an upward tendency In the following survey only cases aged 10–50 were considered

Pulmonary embolisms have so far always been accounted for by an underlying disease Thrombosis connected with the circle of Willis was noticed in five instances the two women already described one woman with atrial thrombosis and endocarditis and two men aged 37 and 45

Cerebral hemorrhages not accounted for by arterial hypertension were reported in five women two were 49 years of age one was pregnant in the 3rd month one had a congenital syphilis and one aged 27 died in connection with sexual intercourse whether contraceptives had been used in those instances is not known

Addendum Whilst reading the proof sheets we have been informed by Tom Saldeen M D Department of Forensic Medicine about a woman, aged 26 who died in the Out patient Department of the University Hospital Uppsala, on Feb 9 1969 She had taken oral contraceptives for six weeks only and now she died from pulmonary embolism there was still a solid thrombosis in the right iliac vein as well as 1 dm up in the inferior vena cava.

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PROTEIN BOUND IODINE DURING ANTITHYROID TREATMENT

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Abst act A combination of low protein-bound iodine values and normal basal metabolic rates has been found in some thyrotoxic patients treated with antithyroid drugs. Experiments are presented which show that this observa tion can be explained by an increase in the triiodo-thyronine share of the circulating thyroid hormones in these patients

The finding of a low PBI value in an antithyroid treated patient will in some cases not be an indication of myxoedema. It is suggested, however that a low PBI value also in these patients is a sign of overtreatment. The practical outcome of these experiments and considera tions, consequently is that measurement of BMR values alone does not ensure adequate control during treatment with antithyro d agents. A determination of PBI values should be included and it is sug,ested that the dose of antithyroid compound is adjusted to keep the PBI within normal limits.

The treatment of hyperthyroidism with anti thyroid thiourea compounds is usually simple to carry out In several cases it is difficult however to decide when a condition of so-called euthyroid ism has been achieved

Over the past three years we have made the observation that some patients treated with anti thyroid drugs may have low protein bound iodine (PBI) levels without any clinical signs of myx oedema and with normal or even slightly in creased BMR values

It is well known that propylthiouracil (PTU) inhibits the mono- and diiodination of tyrosine Several workers (1 5 10 12) have found an increase in the glandular ratio of monoiodotyro-sine to diiodotyrosine (MIT/DIT) after giving PTU This block of the conversion of MIT to DIT has been shown by the same authors to result in the expected increase in glandular tri iodothyronine (T_3) relative to thyroxine (T_4)

Triiodothyronine disappears rapidly from the blood and has a metabolic effect which per weight unit is 3–5 times higher than that of thyroxine Obviously a PTU induced increase in the glandular T_3/T_4 synthesis ratio might explain the present observations of low PBI values and a euthyroid state with normal BMR values in propylthiouracil treated patients It has not previ ously been realized that the above mentioned ob-servations concerning the effect of PTU on thy roid tissue might have such clinical implications The present study was undertaken to call atten tion to this fact To assess the hypothesis of an increase in the T_3/T_4 ratio in blood a series of experiments has been performed

MATERIAL AND METHODS

The serum content of labelled T and T (T and T) was measured in 27 thyrotoxic patients. The ratio of T* to the combined amount of T* and T* was determined in serum collected from the patients 24 and 72 hours after a peroral dose of 300 μC ^{125}I.

From 5 to 12 ml serum (dependent upon the PB^{125}I value) were used for analysis. The inorganic iodine was removed by adding about 4 mg/ml of an anion exchange resin (Dowex 1×2, 200–400 mesh) The mixture was shaken for 10 min and centrifuged. The supernatant was adjusted to pH 2.5–3 and extracted twice with the double volume and twice with the single volume of *n*-butanol Inacti e carriers of MIT DIT T_3, T_4, T and KJ (250 μg of each) were added to the combined extracts and the pH was adjusted to 7.5–8 The combined extracts were then distilled in vacuo and the residue was dissolved in methanol–conc ammonia (99 1)

Aliquots of this solution were applied to Whatman no 1 filter paper and run for about 48 hours in an iso-pentanol-6 *N* ammonia (1 1) solvent (one-dimensional descending system)

The iodoamino acids were identified by spraying with Pauly s reagent. The paper chromatograms were finally cut into one centimetre wide strips and their ^{125}I content was determined.

The error of the method was calculated as the standard error of the differences between the double determina

Table I *T_3-ratio and other thyroid parameters in non treated thyrotoxic patients*

Case no	Age	Sex	BMR	PBI	Serum thyroxine (Murphy proced) (4 5–13 5)	T_3 resin test (¬2-34 4)	Serum cholesterol	$\frac{100\ T_3}{T_3+T_4}$ 24 h after dose	$\frac{100\ T_3}{T_3+T_4}$ 72 h after dose
1	46	♀	136	9 1	13 5	36 3	¬50	—	1 4
2	¬9	o	—	10 3	15 7	35 8	210	7 0	6 5
3	52	♀	178	16 4	37 2	50 5	195	0	1¬
4	59	♀	150	10 6	17 2	41 2	256	10 4	6 0
5	57	-	128	9 8	18 3	41 1	265	3 1	4 2
6	74		144	8 0	16 4	44 4	195	6 7	3 0
7	¬0	-	—	8 9	12 4	40 2	185	7 4	3.2
8	54	♀	165	15¬	30 0	55 2	203	7 0	3 9
9	19	♀	133	8 4	14 7	39 2	180	9 3	5 1
10	5?		161	1?0	21 0	49 1	190	5 9	5 0
11	5		—	8 4	13 7	36 4	2¬0	7 2	3 5
1¬	43	♀	157	17 6	36 4	45 9	270	6 8	4 1
13	4	o	16?	14 6	33 6	55 6	180	4 8	4 6
14	54		155	6 4	13 5	35 9	¬30	6 3	3 5
Mean ±1 S D							216±32	6 3±2 6	3 9±1 5

tions (standard error = $\pm\sqrt{\Sigma d^2/2n}$) All steps of the analysis were made in duplicate and the error of the determination of (T* 100) (T*+T*) (the "T_3-ratio") was found to be ±0 83 and ±0 7¬ for the 24 h and the 72 h ratio respectively

When the means of the different groups were compared, Student's *t* test was employed The differences were considered significant if *P* was less than 0 01

The PBI values were determined by the autoanalyzer procedure (normal range 3 5–8 μg 100 ml) Serum thyroxine was determined by the procedure of Murphy (8 11) (normal range 4 5–13 5 μg 100 ml)

The T resin test was performed by the "Triosorb" procedure (normal range 22.2–34 4% (4)) Serum cholesterol was determined according to Wright et al. (14) (normal range 1⁵0–3⁵0 mg %)

RESULTS

Table I shows the results of the determination of (T_3* 100)/(T_3*+T_4*) (the "T_3 ratio") and some other thyroid parameters in 14 non treated thyrotoxic patients The mean values of the T_3 ratio were 6 3 and 3 9 respectively 24 h and 72 h after giving the dose

Table II *T_3 ratio in PTU treated thyrotoxic patients with normal PBI*

Case no	Age	Sex	No of days treated	Total dose PTU (g)	BMR	PBI	Serum thyroxine (Murphy proced) (4 5–13 5)	T_3 resin test (22.2–34 4)	Serum cholesterol	$\frac{100\ T_3}{T_3+T_4}$ 24 h after dose	$\frac{100\ T_3}{T_3+T_4}$ 7? h after dose
15	47	♀	372	81¬	115	6 4	9 1	28 6	229	—	4 7
16	66	o	565	103 7	105	3 1	7 4	26 4	310	12.7	5 0
17	37	♀	81	33 8	122	4 1	7 5	31 6	305	18 7	11 8
18	30	♀	95	33 8	116	3 3	4 5	33 2	¬90	11 0	6 0
8	54	♀	71	42 6	125	7 5	14 0	29 2	255	16 8	9 7
9	19	♀	88	21 9	—	4 2	8 3	30 4	215	12.9	6 7
19	63	♀	927	8 6[a]	—	5 6	9 8	3¬ 2	305	3 7	4 6
7	20	♀	114	36 8	100	6 9	7 3	31 7	250	15 6	8 7
8	54	♀	139	57 8	100	3 6	5 5	25 6	3¬5	16 2	10 4
11	52	♀	56	25.2	—	5 0	7 7	32.6	255	10 1	5 6
2	30	♂	211	2 3[a]	112	4 6	8 1	31 0	285	6 0	3 3
Mean ±1 S D									275±36	12 4±4 8	7 0±2.8

[a] Treated with carbimazole

Table III *T_3 ratio in PTU treated patients with low PBI*

Case no	Age	Sex	No of days treated	Total dose PTU (g)	BMR	PBI	Serum thyroxine (Murphy proced) (4 5–13 5)	T_3 resin test (22 2–34 4)	Serum choles terol	$\frac{100\ T_3}{T_3+T_4}$ 24 h after dose	$\frac{100\ T_3}{T_3+T_4}$ 72 h after dose
20	61	♀	43ʾ	80 3	101	2 4	1 8	27 7	316	—	9 9
21	44	♀	127	43 5	101	2 8	3 2	24 4	295	12.2	15 6
2ʾ	58	♀	87	36 2	120	2 0	3 2	24 0	410	23 8	10 1
23	27	♀	77	13 7	110	1 7	3 8	26 6	235	20 4	15 7
24	32	♀	90	31 9	100	1 5	1 6	18 0	375	19 9	12 2
25	31	♀	74	26 4	108	1 3	1 5	29 2	3‿5	43 0	28 8
10	52	♀	70	30 8	112	1 8	0 8	28 6	375	14 9	10 5
26	54	♀	222	90 0	128	0 9	0 4	24 2	315	49 5	52 0
27	76	♀	186	9 3	—	2 2	1 4	23 2	280	16 3	8 9
24	3‿	♀	258	50 6	96	‿2	2 0	20 2	395	31 9	21 5
1	46	♀	37	20 4	130	1 5	1 6	21 4	400	—	9 4
Mean ± 1 S D									338 ± 57	25 8 ± 13 0	17 7 ± 12 9

[a] Treated with carbimazole

Table II shows that the T_3 ratios were considerably higher in a group of 11 PTU treated thyrotoxic patients with normal PBI values being 12 4 (24 h) and 7 0 (72 h) A comparison with the values in Table I shows that the increase in the T_3 ratios and also an observed increase in the serum cholesterol are significant ($P < 0\ 01$)

The third group consists of another 11 PTU treated patients (Table III) These patients had PBI levels and serum thyroxine values which were definitely below the normal range It is noticed that the T_3 ratios were found to be much higher in this group with mean values of 25 8 (24 h) and 17 7 (72 h) The mean serum cholesterol for this group showed an increase to 338 mg% This value as well as the T_3 ratios differ significantly from the values in Table II ($P < 0\ 01$)

The T_3 ratio was determined both before and during treatment in seven of the above mentioned patients The data of this group are shown in Table IV In accord with the previous results the mean values (T_3 ratio and serum cholesterol) before treatment differ significantly from those found during treatment ($P < 0\ 01$)

Table IV *T_3 ratio before and after PTU treatment*

			Before treatment					During treatment						
Case no	Age	Sex	BMR	PBI	Serum choles terol	$\frac{100\ T}{T_3+T_4}$ ʾ4 h after dose	$\frac{100\ T}{T_3+T_4}$ 72 h after dose	Days of treat ment	Total dose PTU (g)	BMR	PBI	Serum choles terol	$\frac{100\ T}{T_3+T}$ 24 h after dose	$\frac{100\ T}{T_3+T}$ 72 h after dose
8	54	♀	165	15 2	203	7 0	3 9	71	42 6	125	7 5	255	16 8	9 7
10	52	♀	161	1‿0	190	5 9	5 0	70	30 8	112	1 8	375	14 9	10 5
9	19	♀	133	8 4	180	9 3	5 1	88	21 9	—	4‿	215	12.9	6 7
7	20	♀	100	8 9	185	7 4	3 2	114	36 8	100	6 9	250	15 6	8 7
11	52	♀	—	8 4	‿‿0	7 2	3 5	56	25 2	—	5 0	255	10 1	5 6
2	9	♂	—	10 3	‿10	7 0	6 5	211	2 3	11	4 6	285	6 0	3 3
1	46	♀	136	9 1	250	—	1 4	37	‿0 4	130	1 5	400	—	9 4
Mean ± 1 S.D					‿05 ± ʾ4	7 3 ± 1 1	4 1 ± 1 6					291 ± 70	12 7 ± 4 0	7 7 ± 2 6

Tre ted w th carbimazole

DISCUSSION

The relative composition of the thyroid hormones in human plasma has been the subject of several studies (for references see Emrich and Uhl (3)) The usual finding has been that triiodothyronine constitutes about ten per cent or less of the total plasma thyroid hormone content in thyrotoxic patients

In accord with the experiments of Emrich and Uhl (3) it will appear from Table I that there was no relation between the severity of hyperthyroidism and the T_3 ratio The same authors found that the relative share of labelled T_3 decreased with time We found a similar result since the 72 h T_3 ratio in most cases was lower than the 24 h ratio

It has been mentioned above that PTU treatment leads to an increase of the MIT/DIT ratio and also of the T_3/T_4 ratio in the thyroid gland Arosenius (1) found a mean value of 3 50 for the ratio T_4/T_3 in the normal thyroid gland In glands from antithyroid treated thyrotoxic patients the T_4/T_3 ratio was only 1 43

The present experiments have shown that these PTU induced changes in the glandular hormone synthesis are parallelled by an increase in the relative share of T_3 in the circulation

This will result in lower PBI values since T_3 disappears much more rapidly from the blood than T_4 In some of the patients (shown in Table III) the proportion of T_3 in the blood was very igh resulting in abnormally low PBI values $_3$ ratios as high as about 50 were found (patient no 26)

The combination of a euthyroid state with normal BMR and a low PBI level seems well explained by the finding of an increased T_3 ratio Obviously the ratio of the tissue consumption" of T_3 and T_4 cannot be estimated from the blood T_3 ratio It seems reasonable to assume that an increase in the latter is accompanied by a much more pronounced increase in the tissue T_3 ratio

We have not had the opportunity to examine any patients with regular myxoedema induced by PTU Other cases of myxoedema with an ^{131}I turnover pattern of intense TSH stimulation however have been found to have very high T_3 ratios

It is known from animal experiments (12) that an increase in TSH secretion plays a decisive role in producing an increase in the glandular synthetic T_3/T_4 ratio The PTU treatment caused some enlargement in several of the patients shown in Table III suggesting an increased TSH stimulation This may be an indication that these patients (Table III) were on the way to myxoedema a fact which is masked by the euthyroid state and the normal BMR values According to this view a decrease of PBI to values below the normal range may be considered the most sensitive indicator of overtreatment It is consequently advocated that the PTU dose is adjusted to keep the PBI within normal limits A final decision on this point will however involve a determination of the plasma TSH content during PTU treatment in these patients

In their paper on TSH immunoassay Odell et al (9) briefly report on plasma TSH in some patients treated with PTU Of interest in this connection is that some clinically euthyroid patients with low–normal plasma PBI and elevated TSH levels were found They considered this an indication that the mechanisms designed to restore plasma thyroid hormone concentration to normal occur earlier than clinical evidence of peripheral tissue deficiency of thyroid hormone This observation of a disturbance in the pituitary feed back mechanism in such patients makes it reasonable to speculate that our patients (Table III) may have had high plasma TSH levels It has not been clarified why thyroxine has a lower suppressive effect on the pituitary gland in PTU treated patients *Possibly the inhibitory effect of PTU on* peripheral thyroxine deiodination is involved (6)

*Limited information may be obtained by fol*lowing the change in serum cholesterol values during PTU treatment The mean value of the serum cholesterol in the PTU treated patients with low PBIs (Table III) was 338 mg% It is noticed that the patients with the highest T_3 ratios (nos 25 and 26) had serum cholesterol values within normal limits

The above described effects may possibly also be produced by other thyreostatic compounds In the treatise of Munkner (7) on the effect of para aminosalisylic acid on the ^{131}I metabolism some patients are included in whom a combination of normal BMR and low PBI values was found It is reasonable to suggest that these data may be explained in the same way as those of the present PTU treated patients

A combination of normal BMR and low PBI may also be seen in certain patients with low serum thyroxine binding capacity. Dialysis experiments performed by the method of Christensen (2) and T_3 resin tests have shown, however, that the low PBI values in our PTU treated patients cannot be explained by a change in the serum protein binding of the thyroid hormones.

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ISOLATED PULMONIC VALVULAR REGURGITATION

A Report on Nine New Cases

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Abstract Isolated congenital pulmonic valvular regurgitation has been considered to be an extremely rare anomaly The present paper gives a clinical and haemodynamic description of nine patients seven of whom were seen by one Cardiological Unit in the course of five years A constant clinical sign was a diastolic murmur in the second and third left intercostal space Phonocardiograms disclosed occasionally a crescendo–decrescendo murmur which has been described as typical but more often the murmur was simply decrescendo The shape is apparently of little value in judging the severity of the lesion, as mild cases presented either type of murmur A systolic murmur of varying grade has previously been described as a constant finding in pulmonic valvular regurgitation However in one of the present patients there was no such murmur The diastolic murmur was occasionally very faint and may easily be overlooked It is suggested that isolated pulmonic valve regurgitation may occur more frequently than previously believed

Isolated pulmonic valvular regurgitation is a well described clinical entity (12 15 19) It has been considered to be extremely rare (13 16) In 1965 according to Hager et al (8) only 39 cases had been reported in the literature The anomaly is as a rule congenital but may occasionally arise after gonorrhoeal (18) staphylococcal (15) or rheumatic (22) endocarditis The condition is usually benign but may cause severe symptoms (6) A diastolic and a systolic ejection murmur are found in the pulmonic area Intracardiac phonocardiograms have shown that the diastolic murmur arises in the right ventricle (20) Additional confirmation of regurgitation has been obtained angiocardiographically (3) or by use of dye dilution methods (2)

The purpose of the present paper is to suggest that isolated congenital pulmonic valvular regurgitation may occur more frequently than previously believed Nine patients from one Cardiological Unit are described seven of whom were seen during the years 1961–1965

CLINICAL FINDINGS

Clinical data are summarized in Table I. A chest murmur had been accidentally noted in most patients during infancy or childhood. Symptoms were absent in all except one (K. G) who six weeks previous to the examination had experienced tachycardia after fairly heavy exertion. When hospitalized he had slight ankle oedema and auricular flutter He was treated with digitalis but as flutter persisted successful electroconversion was carried out. When seen by the Cardiological Unit he had no symptoms or signs of congestive heart failure

The pulmonic valvular regurgitation was assumed to be of congenital origin No patient had a history of rheumatic fever or prolonged fever of unknown cause The sero-reactions and haemograms were negative and there was no evidence of systemic disease

On clinical examination none had signs of congestive heart failure There were no signs of cardiac enlargement except in one (S F) in whom the apex beat was felt in the 5th left intercostal space in the anterior axillary line A diastolic murmur was always found in the 2nd or 3rd left intercostal space but it was occasionally very faint (patients K F and K. G) It has often been described as low pitched in quality and with a crescendo–decrescendo appearance on the phonocardiogram (1) However this was the case in only two patients (an example is shown in Fig. 1) the others having a short diastolic murmur with its maximum intensity immediately after the 2nd sound (see Fig. 4) Eight patients had a systolic murmur in the pulmonic area The murmur was short, had its maximum early in systole and always ended well before the 2nd heart sound It was usually fairly weak and never exceeded grade 3 (scale 1–6) This murmur is commonly considered to be due to an increased right ventricular stroke volume Jacoby et al (11) call attention to the second heart sound in the pulmonic area. They presented three cases in which the 2nd sound was always widely split during expiration and

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Table I *Pulmonic valve regurgitation Clinical findings*

Pat	Sex	Age	Age murmur detected	Systolic murmur	Diastolic murmur	2nd heart sound in pulmonic area	ECG
J R	♀	12	Infancy	Gr 2	Decrescendo	Split dur insp	RAD
L M	♂	6	4	Gr 2	Decrescendo	Split dur insp	RAD
E W	♂	34	33	Gr 1	Decrescendo	Split dur insp	Normal
A K		21	20	Gr 1	Cresc-decr	Split dur insp	Normal
F N	♂	5	4	Gr 1-?	Cresc decr	Split dur insp	Normal
S F		6	Birth	Gr 2	Decrescendo	Fixed splitting	IRBBB
K F	♀	20	5	Gr 2-3	Decrescendo	Split dur insp	Normal
O S	♀	19	14	Gr 2-3	Decrescendo	Fixed splitting	RAD
K G	♂	53	19	Absent	Decrescendo	Split dur insp	IRBBB

Abbreviations RAD right axis deviation IRBBB incomplete right bundle branch block Murmur scale 1-6

with minimal variation of the splitting during inspiration. This does not however appear to be a constant finding in pulmonic valvular regurgitation as a similar splitting was present in two of our patients only

Electrocardiograms showed right axis deviation in three incomplete right bundle branch block in two and were normal in four patients All had sinus rhythm at the time of investigation in our laboratory

Chest X ray showed slight to moderate dilatation of the pulmonary artery main trunk. In two patients (S F and K G) the dilatation was especially conspicuous (Fig 2) Marked hilar pulsations were often found and are considered to be typical in pulmonic valvular regurgitation (13) Roentgenological enlargement of the right ventricle was described in two patients (F N and S F) Generally speaking the configuration of the heart shadow in the frontal plane may vary considerably from case to case as shown in Figs 2 and 3 No patient exhibited enlargement of the aorta left ventricle or left atrium.

CARDIAC CATHETERIZATION

The haemodynamic findings are seen in Table II The similarity or approximate similarity of the diastolic pressures in the right ventricle and pulmonary artery

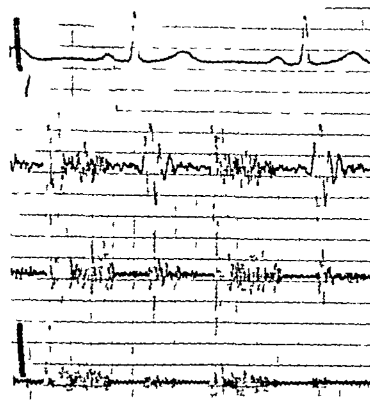


Fig 1 Phonocardiogram showing a crescendo–decrescendo murmur in diastole (patient A K)

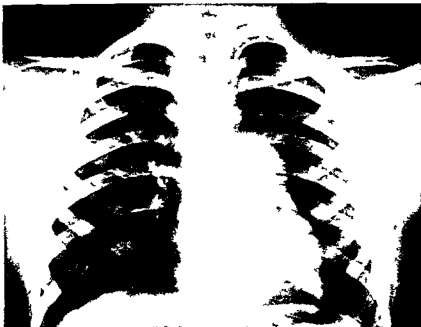


Fig Chest roentgenogram in patient S F Note the dilated main pulmonary artery

should be noted A typical curve is shown in Fig 4 The systolic right ventricular pressure was normal in seven patients borderline in one (O S) and slightly elevated in one (K G) The examination was done under local anaesthesia with the patient supine and zero for pressure recording was the anterior axillary line The oxygen analyses in blood were done spectrophotometrically The saturation values given in Table I are all averages of two or three determinations Occasionally the sensitive hydrogen electrode was employed to exclude left-to-right shunts Cardiac output was estimated by the Fick method Regurgitation from the pulmonary artery to the right ventricle was demonstrated by angiocardiography in one patient (Fig 5)

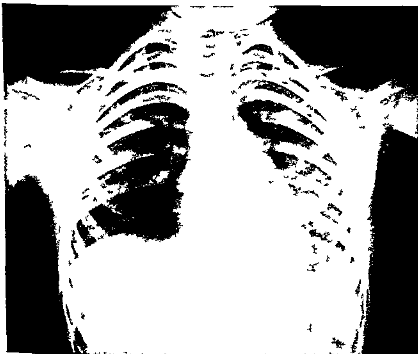


Fig 3 Chest roentgenogram in patient F N Note the heart configuration as compared to Fig 2

Table II *Pulmonic valve regurgitation Catheterization findings*

Pat	Pressures (mm Hg) RA	RV	PA	PAW	BA	Oxygen saturation ( ) SVC	RA	RV	PA	FA	CI	PVR
J R	1	29/-2	19/ 0	4	105/70	68	68		68	100	4 7	194
L. M	0	21/ 1	17/-1	[illegible]	115/80	74	73		75	96	4 5	
E. W	-1	[illegible]8/ 0	22/ 4	1 5	120/70	69	73		73	94	6 0	71
Å K	4	16/ 3	14/ 4	7	130 80	87	81	78	81	99	5 6	48
F N	0	27/-3	22/-0 5	6 5	1[illegible]0 80	62	68	68	65	95	5 2	104
S F	0 5	23/ 0 5	19/ 2 5	2	105/80	68	73	78	69	96	3 1	226
K F	0 5	25/ 2	19 4	7	130 95	72	73		71	94	2 6	91
O S	0	35/-1	23/ 1 5	6	120/80	78	78	77	78	97	3 0	63
K G	4 5	38/ 0	30/ 5	7	140/80	65	72	68	71	95	3 1	106

Abbreviations RA right atrium RV right ventricle PA pulmonary artery PAW pulmonary artery wedge position BA brachial artery (arm cuff) pressure SVC superior vena cava FA femoral artery CI cardiac index ml min/m PVR pulmonary vascular resistance (dyn/sec/cm^{-5})

COMMENTS

As regards the diagnosis blood oxygen analyses or the use of hydrogen inhalation did not show significant shunts in any case Aortic valvular regurgitation was excluded on clinical grounds The systemic blood pressure was normal in all patients There were no clinical signs of left heart enlargement The presence of normal ascending aorta and left heart on the chest radiographs was also a factor contradicting the diagnosis of aortic regurgitation Aortography was not performed as it was felt that the haemodynamic findings corresponding to the clinical and roentgenological signs were reasonably sufficient to support the diagnosis of pulmonary valvular regurgitation

Moreover the pattern of a relatively wide pulmonary artery pulse pressure combined with equal or approximately equal diastolic pulmonary artery and right ventricular pressure shown in Table I was never found among 50 consecutive patients with functional chest murmur seen in our institution

Pulmonic valvular regurgitation in combination with congenital stenosis has been reported (9) and has also been observed in our laboratory However cases in which the additional diagnosis of stenosis might be possible according to the haemodynamic data were excluded from the present material It is considered that all our nine patients had isolated regurgitation as the gradient

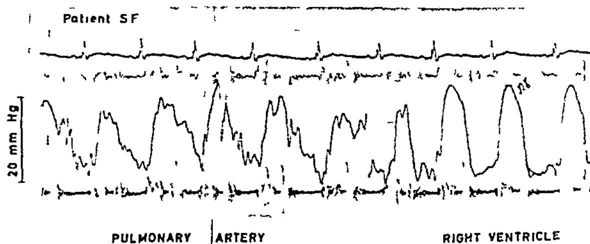


Fig 4 ECG phonocardiograms (pulmonic area) and pressure curve obtained during right heart catheterization on withdrawal of the catheter from the pulmonary artery to the right ventricle Note dicrotic notch" on the descending limb in the pulmonary artery pressure curve corresponding to a decrescendo diastolic murmur

across the pulmonic valve never exceeded 12 mm Hg On the other hand it may be noted that a gradient of up to 40 mm Hg ascribable to "relative" stenosis due to a large amount of blood ejected from the right ventricle during systole has been observed after experimental excision of the valve cusps in animals (5) Similarly a gradient of up to 23 mm Hg has been demonstrated in a patient in whom subsequent operation showed completely destroyed pulmonic leaflets (4)

All our cases were mild and the prognosis in pulmonic valvular regurgitation is generally considered to be favourable This is corroborated by animal experiments in which congestive heart failure did not develop up to 14 months after excision of valve cusps (5) However occasionally a significant increase in heart volume was observed and studies by others have shown a decrease in cardiac output 11–18 months after excision (7)

The case described by Olesen and Fabricius (18) illustrates that pulmonic valvular regurgitation can be tolerated also in humans for long periods as the pressure in the right ventricle as well as the pulmonary artery was 28/0 mm Hg 27 years after the diagnosis was made Also it is generally considered that the development of a diastolic murmur after operation for pulmonic stenosis does not affect the outlook unfavourably Marshall and Jones (17) reported a case with pulmonic valvular regurgitation complicated by thyreotoxicosis The association of these two conditions results in an unusual degree of hyperdynamic activity of the right ventricle However right ventricular end-diastolic pressure remained normal which confirms that the valvular lesion can be well tolerated Most reported cases of isolated pulmonic regurgitation have had no symptoms However two reports of deaths in infancy ascribable to the lesion have been made (10 21) The patients died in congestive heart failure within 30 minutes and three days after birth respectively Ehrenhaft (4) described a 14 year-old boy who had marked cardiac enlargement and who had been symptomatic from the age of 4 years Other authors (8 14) warn that the innocence of pulmonic valvular regurgitation should not be assumed even in the absence of symptoms Hager et al (8) noted that average age of reported patients with mild regurgitation was 18 years while the average age in the severe type



Fig 5 Angiocardiography in patient K. F The catheter tip is placed in the main pulmonary artery Regurgitation into the right ventricle is shown.

was 45 years In the present material only the oldest patient had had symptoms and required digitalization

While little is known about the natural course of the disease it may also be difficult to judge the severity from clinical phono- and electrocardiographic signs Among our nine patients mild cases presented a diastolic murmur either of the decrescendo or the crescendo-decrescendo type Nor was the systolic murmur helpful as the grade in mild cases varied

In one of our patients the systolic murmur was absent This is especially notable as according to one review (8) a systolic murmur had always been present in the earlier reported cases of pulmonic valvular regurgitation

The diagnosis of isolated pulmonic valve regurgitation in nine patients at one Cardiological Unit suggests that the anomaly at any rate in cardiac clinical practice may not be so extremely rare as previously believed The condition may

be overlooked as the physical signs sometimes are very sparse and laboratory investigation is necessary to establish the diagnosis

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URINARY EXCRETION OF FREE AND CONJUGATED HISTAMINE IN VARIOUS GASTROINTESTINAL DISORDERS

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Abst act In order to evaluate the specificity of the in creased urinary excretion of conjugated histamine in myotonic dystrophy patients with various gastrointestinal disorders have been studied. Occasionally the urinary excretion of conjugated histamine in urine was elevated in patients with idiopathic steatorrhoea, coeliac disease and gastritis/peptic ulcer When determined, the hista mine like activity in the stools was augmented in cases with increased urinary output of conjugated histamine

A significantly increased urinary excretion of con jugated histamine was found in cases of chronic pan creatitis The increased faecal histamine like activity in dicated an intestinal origin of the urinary conjugated fraction in this disorder

The quantity of conjugated histamine excreted in the human urine seems at least partly to be a function of the levels of histamine in the lumen of the gastrointestinal tract (6 17 18) Histamine is then conjugated (acetylated) and absorbed (*or vice versa*) and excreted in the urine (17)

Myotonic dystrophy is frequently associated with increased urinary excretion of conjugated histamine (20 21) and in this disorder as well the excess histamine seems to originate from the lumen of the gastrointestinal tract Almost invari ably increased levels of histamine like activity are present in the faeces of these patients (20) and the fundamental aberration in this sequence seems to be increased intraluminal formation of hista mine This indicates that in myotonic dystrophy there is an intestinal dysfunction as well (for reference see (20))

Disorders primarily affecting the gastrointestinal tract are *a priori* the ones most likely to have altered metabolism of histamine in the intestinal lumen Presumably therefore the study of pa tients with such disorders should give some in formation as to the specificity of the histamine abnormalities observed in myotonic dystrophy

The knowledge of intraluminal histamine for mation in gastrointestinal disorders is sparse Faecal excretion of histamine has been studied in patients with ulcerative colitis and was shown to be normal in most cases (13) Some few cases of achylia fermentative dyspepsia, peptic ulcer and diarrhoea have also been studied, and normal or slightly elevated values of faecal histamine were observed (13)

This communication deals with the urinary out put of free and conjugated histamine in a variety of gastrointestinal disorders and for the follow ing reasons particular interest will be paid to steatorrhoea

A moderate increase in urinary excretion of 5 hydroxyindoleacetic acid is frequently found in non tropical sprue and malabsorption (9 11 16) Since increased urinary excretion of 5 hydroxy indoleacetic acid and histamine concur in e g carcinoid syndrome (14) it is tempting to specu late that a derangement of *histamine* metabo lism could also be present in non tropical sprue Moreover serotonin is a liberator of histamine (8)

MATERIAL

All patients in th s study were admitted to hospital How ever some of the patients were studied after dismissal from the hospital

The diagnoses in cases of steatorrhoea were founded on such parameters as faecal fat and nitrogen excretion on standard diet, vitamin A absorption, D-xylose absorp tion, glucose tolerance presence of tryps n in duodenal juice and histology by jejunal biopsy X ray study of the small bowel, secretin stimulation, serum folic acid levels,

Table I *Histamine excretion in urine in healthy children*

Subject no	Sex	Age	Histamine in urine (μg base/24 h) Free	Conjugated
I	♀	2 5	6	5
II	♂	3 5	5	7
III	♀	5	8	10
IV	♀	5	11	—
V	♀	6	3	0
VI	♀	7	2	5
VII	♀	7	5	4
VIII	♂	9	2	5
Mean			5 3	5 1

and S hilling tests In a few cases in which the diagnosis remained uncertain either a descriptive diagnosis is given or a question mark is attached to the diagnosis All patients in Table II had steatorrhoea at the time of study No grading of the steatorrhoea was attempted

A series of eight healthy children forms a control group to the two children with coeliac disease

The patients were usually taken off medication prior to examination Some few patients however still received various drugs which are listed in the tables With two exceptions patients with idiopathic steatorrhoea received gluten free diet. The effect of administration of a gluten free diet on the urinary output of histamine was studied in one patient with idiopathic steatorrhoea (case no 7)

METHODS

For the evaluation of histamine of gastrointestinal origin three parameters were used urinary excretion of conjugated histamine histamine like activity in faeces, and *in gastric and duodenal juice*

For the estimation of free and conjugated histamine in urine the method of Dunér and Pernow (5) was employed Some minor modifications were made (?2) This method involves adsorption of histamine on a cation exchange column (Amberlite IRC 50) with the subsequent eluation with hydrochlorid acid Histamine was then assayed on a segment of the isolated guinea pig ileum suspended in a bath of Tyrode s solution The additional biological activity that occurred upon acid hydrolysis was designated conjugated histamine Urine was collected in 24 hour periods with 60 ml 1.2 *N* hydrochloric acid added to the flasks which were kept at +4 C during collection With this method the following unweighted values were found in a series of healthy adults (18) free histamine 1.6 μg base/24 hours ± 6 3 (mean ± S D) conjugated histamine 30 0 ± 25 8 μg base/24 hours

The mean recoveries of histamine diphosphate (Nutritional Biochemicals Corp) and N acetylhistamine (Calbiochem) added to urine were 72 and 74 per cent, respectively

Free histamine in the faeces was estimated in accordance with a previously described method (19) The highest value for free histamine in the faeces from healthy individuals was < 0 025 μg base/g wet weight (The value < 0 25 μg b/g wet weight for free histamine reported elsewhere (Table IV reference 19) is a misprint for < 0 025) Mean recovery of histamine diphosphate added to faeces was 60 per cent

Table II *Urinary excretion of histamine in patients with idiopathic steatorrhoea and coeliac disease*

Case no	Sex	Histamine in urine Free	Conj	Ha	Comments
1	♀	12	7	—	
2 A	♀	18	53	—	
B		6	110	—	
3		8	90	—	
4		6	63	—	
5	♀	13	6	—	
6	♀	7	110	—	Untreated steatorrhoea
7 A	♂	31	36	—	Gluten free diet
B		23	14	—	No special diet
8	♂	13	1	0 02	
9	♀	10	36		
10	♂	12	60	0 16	
11 A	♀	60	380		Acute relapse
B		26	770	26	During recovery
12 A	♀	1100	0	3 6	
B		130	36	0 7	
13	♀	2	3	—	Child with coeliac disease
14	♀	7	400	3–43	Child with coeliac disease
Weighted normal values (mean and range)		14 (2 31)	28 (1–130)	0 025	

= Faecal histamine like activity (μg base/g wet weight) in corresponding faecal samples
= Highest observed value

Table III *Urinary excretion of histamine in patients with various gastrointestinal disorders*

Case no	Sex	Diagnosis	Urinary histamine (μg base/24 h) Free	Conjugated	Drugs during study
15 *A*	♀	Gastritis	16	230	
B	♂	Gastritis	15	90 (HA 0 15)	
C	♀	Gastritis	25	220 (HA 6 0)	
16	♀	Folic acid depletion Rheumatoid arthritis. Duodenal ulcer	1	2	
17 *A*	♂	Gastric ulcer	37	90	
B	♀	Gastric ulcer	14	26	
18	♂	Gastrectomy	6	150	
19	♀	Di erticulosis	21	30	
20	♀	Whipple s disease Secondary steatorrhoea	20	52	Phenergan®
21	♀	Whipple s disease?	12	44	Durabol
2	♂	Gastrectomy Secondary steatorrhoea	8	34	
23 *A*	♀	Reticulosarcoma Secondary	13	125	
B	♀	steatorrhoea	17	36 (HA traces)	
24	♀	Diabetes Sarcoidosis Secondary steatorrhoea	4	41	
25	♀	Steatorrhoea Hypercholesterolaemia	3	9	
6 *A*	♀	Acrodermatitis enteropathica	7	14	
B	♀	(Danbolt–Closs)	1	19	
27 *A*	♀	Terminal ileitis	22	88	
B	♀	Terminal ileitis	15	86	
28	♀	Perforated append citis	37	48	Various antibiotics
9	♀	Ulcerative colitis	10	6	
30	♀	Ulcerat ve colitis	15	68	
31	♀	Ulcerative colitis	35	9	Cortisone
32	♂	*Ulcerative colitis*	*12*	*4*	
33	♂	Chronic pancreatitis	30	2 0 (HA 0 4)	
34	♂	Chronic pancreatitis	56	980 (HA 27–38)	
35	♂	Chron c pancreatitis	8	600	
36	♂	Chron c pancreatitis	34	190	
Weighted normal values (mean and range)			14 0 (2 31)	28 (1–130)	

= Histamine l ke activity in faeces, in μg base/g wet weight. (Highest observed control value 0 025 μg base/g.)
= This patient is described in detail elsewhere (23)

Free and conj g ted histam ne in gastric and duod nal ju ce were determined as described in detail elsewhere (20) In a control series the highest values for free and conjugated histamine in gastric juice were 0 03 μg base/ml juice and ≤ 0 018 respectively We have only had opportunity to study one control sample of duodenal juice and found the following values free ≤ 0 6 and conjugated ≤ 0 01 μg base/ml (20) Mean recovery of *small quantities of histamine diphosphate added to gastric* and duodenal juice was 74 per cent

The values for histamine are expressed in terms of the base and represent means of duplicate analyses. No corrections have been made for losses during the extraction procedures An antihistaminic agent diphenhydramine hydrochloride (Allerg n®) was occas onally added during the b ological assay The fact that this counteracted the contractions caused by the eluates and those caused by authentic histamine to the same extent was taken as an indication that the substance tested biologically was identical with h stamine (15)

Table IV *Histamine in gastric and duodenal juice in various gastrointestinal disorders*

Case no	D agnos s	Histamine (μg base/ml) Gastric ju ce	Duodenal juice
12	Id opathic steatorrhoea	Traces	0 0
23	Reticulosarcoma Secondary steatorrhoea	0 13/—	< 0 2/—
24	Diabetes Sarcoidosis Secondary steatorrhoea	< 0 03	0 03
37	Gastrectomy Cholecystopathy	—	< 0 6 0 73
38	Gastric ulcer	0 01/ < 0 03	—

= Exam nation carried out at the time of study I B patient no 12 (see Table II)
= Total histamine i e free + conjugated histamine
= Free histam ne conjugated histamine

RESULTS

The results are presented in Tables I–IV Some of the results listed in Table II derive from chil dren (patients nos 13 and 14) The urinary out put of histamine in children seems to differ from that in adults (1) A control series with healthy children was therefore studied with regard to urinary excretion of free and conjugated histamine (Table I)

The urinary excretion of free as well as con jugated histamine was within the control range in most patients with steatorrhoea (Table II)

In two patients (nos 11 and 12) the urinary excretion of *free* histamine exceeded the upper normal limit whereas in patients 11 and 14 the urinary excretion of *conjugated* histamine was definitely elevated Increased urinary excretion of histamine be it free or conjugated was always associated with increased faecal histamine like activity It is noteworthy that in patient 12 study *A* increased amounts of free histamine in the faeces co-existed with low levels of conjugated histamine in urine the free fraction in the urine being elevated

In cases 6 and 7 (sample *B*) no special diet was ingested but this led to no difference in the histamine pattern

Table III embodies cases of various gastro al disorders It appears that two of five ients with gastritis or present/previous peptic cer (gastrectomy) excreted increased amounts of conjugated histamine in the urine Histamine like activity in the stools was estimated in only one of these two patients and was slightly elevated

On biological assay of urine for free histamine it was shown by the internal standard technique that the eluate from patient 16 (Table III) con tained a heavy antihistaminic activity This pa tient who suffered from rheumatoid arthritis and duodenal ulcer and showed a low serum folic acid was medicated with acetylsalicylic acid 4–6 g per day until 36 hours prior to the beginning of the urine collection

In four patients with steatorrhoea due to chron ic pancreatitis there was significantly increased urinary excretion of conjugated histamine ($P <$ 0 05) Faecal histamine like activity proved to be markedly elevated in the specimens examined (patients 33 and 34)

In the remaining disorders the urinary output of histamine did not exceed the normal range

The histamine concentration of gastric and duodenal juice (Table IV) in various gastrointesti nal disorders was either normal or only slightly above previously established normal limits (7 20)

DISCUSSION

The present study reveals that whereas urinary excretion of conjugated histamine is found oc casionally in patients with idiopathic steatorrho a coeliac disease gastritis and peptic ulcer it seems to be present more regularly in chronic pan creatitis Increased urinary excretion of conjugated histamine is thus by no means specific to myo-tonic dystrophy On the other hand the incidence of increased excretion of conjugated histamine is higher in mytotonic dystrophy than in the afore mentioned disorders except chronic pancreatitis

No definite conclusions as to the origin of the high *free* histamine in cases 11 and 12 can be drawn on the basis of the present data

The association of increased amounts of free histamine in urine with increased faecal histamine in patient 12 study *A* seems to be an exception to the regular pattern which is high concentra tions of histamine like activity in the faeces are accompanied by elevated conjugated histamine in the urine The most plausible explanation for this finding however seems to be the following The method used does not allow accurate estimation of the conjugated fraction in the presence of high amounts of free histamine (for discussion see (18)) Even markedly increased excretion of con jugated histamine could thus be present but not detectable in this case

The urinary excretion of free histamine seems to be a rather sensitive indicator of the total endogenous turnover of histamine (2) Increased urinary excretion of free histamine has only been demonstrated in one case of the Strøm–Zollinger–Ellison s syndrome (4) not in other cases of duodenal ulcer (4)

Several other investigations have a bearing on the relationship between peptic ulcer and hista mine metabolism but there is a conspicuous lack of positive evidence (7 10 12 13 24)

The finding of increased urinary excretion of conjugated histamine in two of five patients with gastritis or peptic ulcer needs further corrobora tion

In ulcerative colitis as well no clear-cut pictu

evolves as for the metabolism of histamine Myhrman and Tomenius (13) found increased histamine levels in the faeces of two of eight patients Binder and Hvidberg (3) found increased histamine contents in the rectal mucosa in 17 biopsies of a total of 46 biopsies from 36 patients They ascribed the increase in histamine content to a co existing allergic condition since eosinophilia was present in all biopsy specimens with increased histamine content The present investigation showed that the urinary excretion of conjugated histamine was within the control range in four patients with ulcerative colitis

The conspicuous finding was the significantly increased urinary output of conjugated histamine in patients with chronic pancreatitis The increased faecal excretion of histamine like activity in this disorder points to a gastrointestinal source of the histamine The pattern thus seems to be the same as in patients with myotonic dystrophy The significance of the observed abnormalities in histamine metabolism in chronic pancreatitis remains obscure Further studies will be carried out to elucidate this problem

ACKNOWLEDGEMENTS

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TREATMENT OF IMMUNOLOGIC DISEASES WITH CYTOSTATICS

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Abstract Cytostatic treatment has been given to 40 patients with immunologic diseases Thirty-one patients had connective tissue diseases, five patients primary renal disease three immune hemolytic anemia and one patient bronchial asthma Remission or improvement were obtained in 3[illegible] of the patients Long term treatment was necessary the average duration of the cytostatic therapy being 14 months range from [illegible] to 50 months Withdrawal of the cytostatic drug without flare up of the disease activity was possible only in 14 out of 3[illegible] patients in whom the cytostatic therapy had been efficient In [illegible] out of 39 patients who had been on long term glucocorticoid medication the steroid therapy could be permanently discontinued. A statistically significant fall was observed in serum gamma globulin, and the titer of rheumatoid factor and LE factor decreased in 23 out of 35 initially positive reactions Four patients died from pancytopenia and sepsis The treatment requires careful supervision of the patients and should only be given on strict indications

The use of cytostatic drugs in the treatment of certain diseases in which immunologic mechanisms are supposed to play an etiological or pathogenetic role has been reported with increasing frequency during the last decade (2 3 4 6 10 13) Via their influence on the lymphocytes and the plasma cells the cytostatics inhibit the immunological reactions connected with the humeral as well as the cellular hypersensitivity (5 7 8 11 12)

Furthermore however the cytostatic drugs are able to inhibit the phagocytosis (7) and the non specific inflammatory and reparative processes in the connective tissue (1)

In 1965 we published our primary results of the cytostatic treatment of 25 pati nts with collagen diseases (10) Below we report a follow up study of these patients and the results from the treatment of a further 15 patients

MATERIAL

Since 1964 we have given cytostatic treatment to a total number of 40 patients suffering from various immunologic disorders Thirty-one of the patients had connective tissue diseases (Table I) five patients had primary renal diseases three immune hemolytic anemia and one patient bronchial asthma From Table I it appears that 16 patients had symptoms of renal disease In these patients the renal component played a prominent role in the disease

All patients had chronic active diseases with disabling symptoms demanding treatment. The average duration of the disease before start of cytostatic therapy was 30 months (Table II) Other therapeutic possibilities had been exhausted

In all patients but one long term treatment with glucocorticoids had been necessary This treatment had either been insufficient or involved serious side effects Several attempts to withdraw the steroid therapy had been followed by a flare up of the disease

Laboratory investigations

All the patients had the following blood examinations during treatment hemoglobin white blood-cell count, platelet count, reticulocyte count and erythrocyte sedimentations rate (ESR) at one or two-week intervals Every four weeks we estimated serologic reactions for rheumatoid factor and LE factor (Table VI) and determined the concentration of the serum albumin alpha alpha$_2$ beta and gamma globulin (paper electrophoresis) All patients with renal disease had kidney biopsy before the start of the cytostatic treatment and six of these patients had repeated biopsies at different intervals after the start of therapy (A detailed study of the effect of the cytostatic therapy in the patients with renal disease is in preparat on) The kidney function was studied by determination of serum creatinine once a week and creatinine clearance at two-week intervals Microscopic examination of urinary sediment and quantitative determination of urinary protein were performed once a week

METHOD

Nearly all patients were treated with the purine analog azathioprine (Imurel® (suppl ed by Burroughs Wellcome

Table I *Treatment of immunologic diseases with cytostatics*

Disease	No of pats	Comments
Rheumatoid arthritis	1?	8 stage II 4 stage III 2 patients also glomerulonephritis
Systemic lupus erythematosus	8	4 patients with renal disease
Polyarteritis	3	3 patients with renal disease
Polymyalgia rheumatica	3	1 patient with temporal arteritis
Scleroderma	1	
Unclassified collagen disease	4	2 patients with renal disease
Primary renal disease	5	
Immune hemolytic anemia	3	
Bronchial asthma	1	
Total no	40	16 patients with renal disease

Co)) which is an antimetabolite suppressing the synthesis of purine nucleotide Some patients were treated with the closely related purine analog 6-mercaptopurine (Purinethol®) at the start of the therapy and were later changed to azathioprine The dose of azathioprine was 3 mg kg body weight for ten days followed by 1 5-2 0 mg kg as a maintenance dose The dose of 6-mercaptopurine was half as much All patients but one were under treatment with glucocorticoid at the start of the cytostatic therapy In about half of the patients (Table II) a permanent withdrawal of the steroid therapy was possible in the other patients a combined therapy with glucocorticoid and cytostatic drug was necessary The duration of the treatment was determined by the clinical and laboratory signs of disease activity alone

RESULTS

The duration of the cytostatic therapy was rather long the average duration for all patients being 14 months (Table II) Clinical improvement (alterations in the general condition body temperature symptoms from joints and muscles and changes in the laboratory findings characteristic of the diseases) was observed in most of the patients (Table III) Remission (disappearance of signs of disease activity) was seen in ten patients In only four patients—two with SLE one with polyarteritis and one with scleroderma—did the disease progress in spite of the therapy All four patients were in advanced stages of the diseases with severe impairment of renal and lung function Half of the patients with renal diseases improved under the cytostatic therapy (Table IV) The erythrocyte sedimentation rate the serum

Table II *Duration and treatment of diseases*

Disease	Duration of disease[a] (mo)	Cytostatic only	Cytostatic+ prednisone	Duration of therapy[b] (mo)
Rheumatoid arthritis	69 (6-168)	10/12[d]	?/12[d]	18[c] (½-43)
Systemic lupus erythematosus	63 (1 144)	5/8	3/8	22 (3-50)
Polyarteritis	10 (2-17)	2/3	1/3	3 (-4)
Polymyalgia rheumatica	7 (1 12)	2/3	1/3	11 (5 16)
Scleroderma	12	0/1	1/1	26
Unclassified collagen disease	68 (36-132)	2 4	2 4	7 (2-13)
Primary renal disease	4 (3-60)	0 5	5/5	15 (10-?4)
Immune hemolytic anemia	31 (2-60)	1/3	2/3	23 (12 ?3)
Bronchial asthma	48	0 1	1/1	1
All patients	30 (1-168)	22/40	18/40	14 (½-50)

[a] Duration of disease at initiation of therapy
[b] Duration of therapy as of January 1968
[c] Mean and range
[d] Number of patients treated out of total number

Table III *Clinical response to treatment with cytostatics*

Disease	Remission	Improvement	Unchanged no progression of disease	Progression of disease
Rheumatoid arthritis	4/12	7/12	1/12	0 12
Systemic lupus erythematosus	1/8	4/8	1/8	2 8
Polyarteritis	0 3	2/3	0 3	1/3
Polymyalgia rheumatica	2/3	0 3	1/3	0 3
Scleroderma	0/1	0/1	0/1	1/1
Unclassified collagen disease	1/4	3/4	0 4	0 4
Primary renal disease	0/5	5/5	0/5	0 5
Immune hemolytic anemia	2/3	0/3	1/3	0/3
Bronchial asthma	0/1	1/1	0/1	0/1
All patients	10 40	22/40	4 40	4 40

Table IV *Patients with chronic renal disease treated with a cytostatic*

Disease	No of pats	Improvement	Unchanged	Progression of disease
Chr glomerulonephr	7	4+2[a]	1	(2)
Ac glomerulonephr	1	0	1	0
Lupus nephritis	4	2	0	2
Polyarteritis	3	1	0	2
Idiopathic nephrotic syndrome	1	1	0	0
Total no	16	8+2	2	4-2

[a] In two patients the improvement was transient

Table V *Changes in ESR and serum proteins following treatment with cytostatics*

	ESR	Albumin	Alpha 2 glob	Gamma glob
Initial values	76 9+6 5 (40)	2 96±0 10 (40)	0 80+0 03 (40)	1 50+0 10 (40)
Changes in per cent of init values	−39 8±6 4[b] (38)	25 3±5 5[b] (35)	−10 0±4 2[c] (35)	−16 7±3 9[b] (35)

[a] Mean ± standard deviation of mean Number of patients tested is given in parentheses
[b] Statistically different from zero at 0 1 level.
[c] Statistically different from zero at 2 5 level

alpha globulin and the serum gamma globulin fell whereas the serum albumin rose following the treatment (Table V) Of 35 positive reactions for rheumatoid factors and LE factor 23 became negative (Table VI) From Table VII it appears that a permanent withdrawal of the cytostatic therapy was possible only in 14 of the 32 patients in whom the cytostatic therapy had been beneficial Withdrawal of therapy was not possible in any of the patients with primary renal disease

The side effects of the cytostatic treatment are listed in Table VIII Half of the patients had moderate dyspeptic complaints (nausea vomiting) particularly during the first two weeks of treatment disappearing after reduction of the dose of the cytostatic drug Symptoms of severe colitis as described previously in one patient (10) were not observed in other patients A transient minor fall in hemoglobin was observed in one third of the patients within the first four weeks of the treatment A pronounced fall in the white blood cell count and in the platelet count occurred in four and seven patients respectively (Table VIII) Apart from the four fatal cases the changes in

Table VI *Changes in rheumatoid and antinuclear factors following treatment with cytostatics*

Test	Before treatment	After treatment			
	Positive	Negative	Decreased	Increased	Unchanged
Rose Waaler	8	2	3	0	3
Latex fixation	9	6	0	0	3
Streptococcus agglutin titer	8	4	0	0	4
Antinuclear factor	7	2	4	0	1
LE cell	3	2	0	0	1
Total	35	16	7	0	12

Table VII *Patients in whom withdrawal of cytostatic therapy was possible*

Disease	No of pats	Observation period after withdrawal of therapy (mo)
Rheumatoid arthritis	6/11[a]	21 (8-38)[b]
Systemic lupus erythematosus	3/5	20 (3-36)
Polyarteritis	0 2	
Polymyalgia rheumatica	2 2	30 (25-36)
Scleroderma	0 0	
Unclassified collagen disease	1/4	35
Primary renal disease	0 5	
Immune hemolytic anemia	2 2	?6 (25- 6)
Bronchial asthma	0 1	
All patients	14 32	26 (3-38)

[a] Number of patients in whom withdrawal was possible out of total number
[b] Mean and range

Table VIII *Side-effects during treatment of 40 patients with cytostatics*

Side-effect	No of pats
Dyspepsia	4
Anemia, transitory	14
Leucocytopenia	
<2000 per mm³	6
<1000 per mm³	4
Thrombocytopenia	
<100 000 per mm³	5
< 50 000 per mm³	7
Infections	6
Deaths	4

white blood cells and in platelets were transient disappearing after temporary discontinuance of the treatment Infections of different types were seen in six patients Four died in a state of sepsis and pancytopenia Two of these patients have been reported previously (10) In three of the patients the lethal complications occurred within the first four weeks after the start of treatment and during the withdrawal of long term treatment with glucocorticoids

DISCUSSION

The spontaneous course of the immunologic diseases subjected to cytostatic therapy in the present investigation is characterized by a tendency to remission particularly in the early stages of the diseases This impedes the interpretation of the effect of the treatment In our opinion however there is no doubt that the cytostatic therapy is responsible for the improvement observed in three-quarters of the treated patients All patients showed signs of chronic active disease and most of them had been given long term treatment with glucocorticoids before the start of the cytostatic therapy Several fruitless attempts had been made to withdraw the steroid therapy The cytostatic therapy made permanent withdrawal of the steroid therapy possible in half of the patients Furthermore reduction or withdrawal of the cytostatic therapy in about half of the patients in whom the treatment had been efficient resulted in a flare up of the disease (Table VII)

The improvement observed in 75% of the treated patients is in accordance with our previous results (10) and with those of other authors (2 4 6 13) From the present study it appears that the treatment with cytostatic drugs like the treatment with glucocorticoids is a long term treatment This suggests that in all probability the cytostatic treatment as well is only symptomatic It seems reasonable to assume that the effect of cytostatic drugs on the diseases in our patients is due to

an inhibitory effect on the immune mechanisms in the body Most evidence indicates that im munologic phenomena play a pathogenetic role in these disorders (14) and the inhibitory effect of cytostatic drugs on the immune mechanisms is well documented The fall in the sedimentation rate and in the serum alpha globulin as well as the increase in the serum albumin merely reflects a decrease in the disease activity So may also the fall in the serum gamma globulin This fall might, however be due directly to the cytostatic treatment Despite a decrease in rheumatoid fac tors and LE factor in serum in two-thirds of the patients no convincing correlation was found be tween these changes and the clinical improvement This observation is in accordance with the re sults of other authors (15) and is not surprising in the light of the questionable pathogenetic im portance of these factors Possibly the most im portant effect of the cytostatic drugs is the in hibitory influence on the cellular hypersensitivity (14) Finally however the inhibitory effect of the cytostatics on the non specific inflammatory and reparative processes in the connective tissue might be of importance for the improvement of the connective tissue diseases

In 20 of the patients the cytostatic therapy was unable to induce remission or improvement (Table III) This seems to be due primarily to the advanced stage of the disease

Like other authors we have observed an ap parent synergistic effect between cytostatic drugs and glucocorticoids (13) Possibly the two kinds of drugs act in different sites in the immune reac tions (16) Combined therapy may therefore prove more efficient than cytostatic therapy alone Furthermore combined therapy might allow a reduction of the dose of either drug and thereby decrease the incidence of the side effects

The four deaths emphasize the serious com plications which may result from the treatment with cytostatics The long term glucocorticoid therapy which these patients had received previ ously was in all probability a contributory cause of the fulminant septic course Patients who have received long term steroid therapy are probably particularly sensitive to the depressing effect of the cytostatic drugs on the bone marrow espe cially during withdrawal of the glucocorticoids

Apart from the side-effects listed in Table VIII the chromosomal abnormalities which may be induced by cytostatic drugs have to be taken into consideration (9)

It may be concluded from our studies and from the literature that cytostatic drugs are able to induce improvement in immunologic disorders However the treatment seems primarily to be symptomatic and the influence on the prognosis remains to be elucidated

Because of the risk of serious side effects the indications for therapy with cytostatics should be very strict and be limited to serious diseases refractory to other therapeutic procedures The treatment requires a thorough knowledge of the cytostatic drugs used and the most careful super vision of the patients

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CARDIOVASCULAR STUDY OF 100 CHRONIC ALCOHOLICS

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Abstract Clinical ECG X ray and laboratory findings are reported in a series of 100 male chronic alcoholics aged from 23 to 63 years and hospitalized for psychiatric and social reasons Eighty per cent of the patients were periodic drinkers and in 80 per cent alcoholism had lasted for more than 10 years.

Although palpitation extrasystoles dyspnea and chest pain were usual complaints no definite cases of alcoholic cardiomyopathy were found The ECG abnormalities recorded were of about the same order as found in other groups of Finnish men of the same age except for sinus tachycardia (21 per cent) and prolongation of the Q-T time (7 per cent)

One third of the patients had chronic cough 17 had signs of old tuberculous lung disease 5? had enlargement and or tenderness of the liver Anemia and hypomagnesemia were recorded in 55 and 42 per cent, respectively

Failure to detect cardiomyopathy in this study is considered to be due to the low incidence of specific heart disease in alcoholics or perhaps to the periodic type of drinking with poor caloric intake during the drinking bouts in most of the patients in the present material.

An association between excessive alcohol consumption and heart disease has been suspected for a long time (7) In 1929 cardiac manifestations of Oriental beriberi were described (1) Thereafter beriberi was also accepted as the cause of heart disease in alcoholics as excessive alcohol intake with marked nutritional deficiency was found to produce thiamin deficiency and thiamin treatment often had a dramatic effect on the high output heart failure of these patients

During the last decade increasing interest has been directed to heart disease of unknown origin In this connection the importance of alcohol as a possible cause has been revived (10 11 12) In contrast to beriberi heart disease this type of heart failure is characterized by normal or low output and there is no response to thiamin A toxic effect of alcohol on the myocardium has been suggested and experimental (19 31 37) and clinical (42 43) evidence has been presented supporting this view In the opinion of several authors a special form of alcoholic cardiomyopathy can be distinguished from other cardiomyopathies (3 4 13 15 21)

Recently the regional occurrence of acute heart failure and pericardial effusion in beer drinkers has been reported from different parts of the world (26 32 33) Obviously this type of heart disease is at least partly caused by substances added to the beer such as cobalt and is not due to alcohol alone (34)

The idea of alcoholic cardiomyopathy is based mainly on the frequent occurrence of alcoholism as an anamnestic feature in patients hospitalized because of heart disease of unknown origin and on the favorable effect of alcohol abstinence on the course of the disease In the present study 100 heavy drinkers hospitalized for psychiatric and social reasons were examined in order to evaluate the frequency of heart diseases in chronic alcoholics including cardiomyopathy i e congestive heart disease cardiac enlargement or electrocardiographic abnormalities not due to coronary hypertonic or valvular heart disease

MATERIAL AND METHODS

One hundred male alcoholics were studied during the spring of 1967 The patients were examined within two days after admission to the special unit for alcoholics at Hesperia Hospital Helsinki. All admissions were made during or immediately after a heavy bout of drinking. In [illegible] patients the psychiatric diagnosis was delirium tremens, but the others had no psychotic signs Most of the patients were physically and socially in a bad state 37 of them were without permanent lodging and had lived mostly outdoors during the previous weeks All patients were hospitalized voluntarily and most of them because they wanted to stop the drinking bout. The age range of the patients was 23 to 63 years (mean 44 6)

Table I *Age distribution and social status of 100 chronic alcoholics*

Age distribution		Occupation		
Range	*No of pats*		*Formerly*	*During the last year*
0– 4	1	Office		
5 29	3	workers	23	18
30–34	10	Skilled		
35 39	18	workers	50	31
40–44	15	Unskilled		
45–49	[illegible]	workers	47	41
50–54	15			
55 59	[illegible]	Unemployed	—	10
60–64	4			

Physical examination was carried out by two of us and special attention was directed to signs suggesting heart and liver disease Anamnestic data concerning social status, alcohol consumption drinking habits food intake smoking previous illnesses and symptoms of cardiac and other diseases were obtained by questioning the patients Data characterizing the present material are summarized in Tables I and II

A twelve lead ECG was taken with a six-channel Siemens Cardioscript. The ECGs were analyzed using the Minnesota Code (6) The alterations of the original code in regard to S–T segment changes as recommended by the Research Committee of the International Society of Cardiology (23) were taken into account The age group 40–59 years was analyzed separately to facilitate comparison with other population studies in Finland Special care was taken to detect T wave changes such as cloven spinous and dimple T waves suggested by Evans (13 14) as being specific of alcoholic heart disease A T wave index—the ratio of T height (in mm) to T width (in msec) at a level half of T s height—exceeding 100 was arbitrarily chosen to indicate peaked T waves Drugs which might have influenced the T waves were withheld until the ECG had been taken

An antero-posterior and lateral X ray picture of the chest was taken on 100-mm film Blood samples were drawn for determination of the red-cell sedimentation rate hemoglobin hematocrit iron potassium bromide and enzymes The results of enzyme studies are published separately (8)

The intrinsic heart rate was determined in all patients with sinus rhythm by combined parasympathetic and beta receptor blockade with atropin (0 04 mg kg, maximal dose 2 4 mg) and propranolol (Inderal® 0 1 mg/kg) The drugs were drawn into the same syringe and administered i v (17)

RESULTS

The most often recorded subjective cardiac complaint in chronic alcoholics was palpitation and/or extrasystoles occurring especially during the hangover period (Table III) Forty three patients had chronic or periodic dyspnea Several patients had also experienced chest pain but only eight were considered as likely to have real effort angina One of them had been hospitalized previously because of myocardial infarction

The incidence of pulmonary disease was also rather high Smoking was a common habit and one third of the patients had chronic cough Ten patients had been treated previously for tuberculosis of the lungs Symptoms suggesting gastritis were frequent and five men had been operated on because of perforated or chronic ventricular ulcer Two other men had had operations because of lung and rectum cancer respectively Another patient was later found to have a brain tumor

The most frequent clinical finding in chronic alcoholics was enlargement and/or tenderness of the liver (Table IV) There was a relatively high incidence of systolic ejection type heart murmur probably due to increased cardiac output In four cases the loudness of the murmur and its radiation to the neck suggested aortic stenosis A triple rhythm due to a third heart sound was heard in four patients Slight elevation of the systolic and diastolic blood pressure was a common finding but only seven patients had signs of left ventricular hypertrophy in the ECG Two of the patients had pitting edema of the ankles In one

Table II *Type of alcoholism*

Duration (y)	No	Drinking habit	No	Type of alcohol	No	Patients also drinking denatured spirits	Last drinking bout	No
1 5	11	Continuous	20	Distilled spirits	11	9	< 1 week	9
5 10	9	Periodic	80	Wine and beer	9	18	1–4 weeks	36
>10	80			Wine beer and pirits	[illegible]	[illegible]	4 weeks	55

Table III *Anamnestic data of 100 chronic alcoholics*

Palpitation and or extra systoles	Dyspnea	Effort angina	Smokers	Chronic cough	Old lung tuberculosis	Surgery because of ventricular ulcer
73	43	8	91	32	10	5

Table IV *Clinical findings in 100 chronic alcoholics*

Enlargement and or tenderness of the liver	Systolic ejection type heart murmur		Blood pressure mm Hg			
	Total	Suggesting aortic sten	Systolic 150–170	≥175	Diastolic 101–110	>110
52	34	4	37	14	14	10

case it was probably of hepatic origin In the other case there was no obvious cardiac or hepatic cause

Practically all of the periodic drinkers maintained that they ate very little or not at all during their drinking bouts but only few patients were underweight.

The ECG findings are shown in Table V The most often recorded finding was sinus tachycardia, which was observed in 21 cases It occurred as a single finding in eight patients all of them in the age group 40–59 years With the exception of the prolonged QT time (7 cases) the occurrence of ECG abnormalities covered by the Minnesota Code (6) was about the same as in other groups of Finnish men of the same age (?7 36) Using the T wave criteria of Evans (14) two cases of cloven" T wave were found There were no dimple or spinous T waves in this material A T index >100 suggesting peaked T waves occurred in 16 cases (11 in the age group 40–59)

Simultaneous injection of atropin and propranolol was tolerated well by all 97 patients tested Ninety-one patients had an intrinsic heart rate within the normal regression for this drug dose (17) In four cases the heart rate was elevated and in two cases *slightly* depressed In 17 of the 21 patients with sinus tachycardia the heart rate was lowered by the blockade

The X ray pictures revealed definite cardiac enlargement in one case only This was a patient with old myocardial infarction and atrial fibrillation Moderate prominence of the left or right ventricle was found in nine cases Fibrosis of the lungs and pleural adhesions were quite common In addition to the ten cases with a previous history of tuberculous infection seven further cases were found with calcified apical changes suggesting old tuberculosis

Abnormal laboratory findings are summariz d in Table VI Every second patient had hemoglobin and hematocrit values suggesting anemia (44) and one third of them had low serum iron values Forty two of the patients had hypomagnesemia Hypokalemia occurred less often In nine pati¬nts

Table V *ECG findings in 100 chronic alcoholics*

		Total material	Age group 40–59
Number		100	64 (64)
Normal		38	22 (34 4)
With findings		62	42 (65 7)
Minnesota Code No			
I	Myocardial infarction	3	2 (3 1)
IV	ST depression	2	1 (1 6)
V	Negative or flat T waves	10	5 (7 8*)
II_1	Left axis deviation	1	1 (1 6*)
III	High R waves	7	4 (6 3)
VII_1	LBBB	2	1 (1 6)
$VIII_3$	Atrial fibrillation	2	2 (3 1)
	Nodal rhythm	1	1 (1 6)
	Sinus tachycardia	21	13 (0 2)
IX	Low voltage	4	3 (4 7)
	High T waves	8	6 (9 4*)
	Prolonged QT (>10)	7	5 (7 8)

Table VI *Abnormal laboratory findings in 100 chronic alcoholics*

ESR > 30 mm h	Hb < 14 g 100 ml	Hcr < 4?	Serum Fe < 50 μg 100 ml	Serum K < 3 8 mEq l	Serum Mg < 1 8 mg/100 ml
8	55	49	15	7	42

the serum bromide concentration suggested consumption of sleeping pills containing bromide

DISCUSSION

Although non specific signs like tachycardia palpitation chest pain and shortness of breath were common complaints in chronic alcoholics in the present material no definite cases of beriberi heart disease or alcoholic cardiomyopathy were found Analysis of the ECGs did not reveal any increment of arrhythmias intraventricular blocks or those T wave changes considered to occur quite often in connection with alcoholic cardiomyopathy (3 11 13 14)

Except for sinus tachycardia and prolongation of the Q–T time the ECG findings were of the same order as found in studies of two rural populations in Finland (27) and in a study of policemen in Helsinki (36) Sinus tachycardia was the most frequent finding as stated also by other investigators (3 16) It was not due to anemia since out of the 21 patients with tachycardia only three were among the 17 patients with hemoglobin values of less than 12 5 g Sinus tachycardia after alcohol intake is probably caused by acetaldehyde the main degradation product of ethanol As shown by James and Bear (24) perfusion of the sinus node with ethanol slows the sinus rate in dogs whereas acetaldehyde in concentrations comparable to those occurring in man causes sinus tachycardia This accelerating effect seems to be mediated by catecholamines as it can be blocked with propranolol and is absent after reserpinization (24) Although the dose of propranolol employed in the present study was possibly not large enough to cause complete beta receptor blockade (17) sinus tachycardia was depressed in 17 cases out of 21 by simultaneous injection of atropin and propranolol The importance of catecholamines for the occurrence of sinus tachycardia in alcoholics is supported hereby

Combined sympathetic and parasympathetic blockade has been suggested by Jose (25) for the recognition and assessment of heart disease No patients with significant depression of the intrinsic heart rate were found in this material but for safety s sake a smaller dose of propranolol was used than recommended by Jose

Relative prolongation of the Q–T time by more than 10 per cent of the normal a rare finding in population studies was present in 7 per cent of the alcoholics It was possibly due to electrolyte disturbances since two of the patients had hypokalemia (serum K < 3 6 mEq/l) two had hypomagnesemia (serum Mg < 1 8 mg/100 ml) and one patient had both Electrolyte disturbances like combined hypocalcemia and hypokalemia or hypocalcemia and hypomagnesemia cause prolongation of the Q–T time (41) It has also been suggested that hypomagnesemia might cause loss of the intracellular potassium by reduction of the activity of the magnesium-dependent enzyme adenosine triphosphatase thus leading to a prolongation of the Q–T interval (30) Low serum magnesium values are a common finding in chronic alcoholics (20) and were present in 42 patients in this material Correlation between hypomagnesemia and prolongation of the Q–T time was poor since only three of these patients had a prolonged Q–T time According to Surawicz (41) hypomagnesemia alone does not cause ECG changes

Tachycardia and relative prolongation of the S–T interval—the T–P phenomenon —is emphasized as common in alcoholics by Alexander (3) It is found in alkalosis from any cause e g from hyperventilation (2) In the present material hyperventilation was quite common during the hangover period A T–P phenomenon was present in 12 cases all of them among the 21 patients with sinus tachycardia Only one of them had real prolongation of the Q–T time

The occurrence of a brisk ECG with peaked T waves in chronic alcoholics was emphasized by

Levine et al (29) In the present material a T wave index greater than 100 suggesting narrow and peaked T waves was found in 16 out of 100

The paucity of specific ECG abnormalities referable to alcohol in the present material agrees well with other studies on chronic alcoholics selected on account of their alcoholism and not on account of heart disease (5 16 39) Only Priest et al (35) who studied 37 psychiatric in patients with alcoholism were able to record T wave changes in 15 of their cases suggested by Evans (14) to be specific of alcoholic cardiomyopathy Other authors (11) have been reluctant to attach any significance to such T wave abnormalities Non specific changes of the S–T segment and the T wave are known to occur in sympathicotonic states (22) and can be normalized by beta receptor blockade (18) There were only two cases with cloven T in the present material no spinous or dimple T's were found In another report (38) of 164 chronic alcoholics 59 of whom had cirrhosis of the liver ECG findings included practically only T wave changes Forty-one patients had flattening of T waves and nine had negative T waves suggesting left ventricular strain

The fact that no definite cases of alcoholic heart disease were found in the present material needs further consideration The patients were selected on account of psychiatric and social causes but no negative selection of patients with cardiac complaints was made by the doctors responsible for hospitalization It is also improbable that alcoholics with heart disease would have attended outpatient departments of other hospitals Chronic alcoholics in the city of Helsinki are accustomed to seek help for all kinds of symptoms at the special outpatient department of Hesperia hospital where treatment is offered free of charge to them Natural selection by death hardly explains the absence of alcoholic cardiomyopathy provided that the disease does not run a very rapid downhill course

There remains the question of the severity and duration of the alcoholism in the present material The importance of continuous alcohol consumption for the occurrence of alcoholic cardiomyopathy has been stressed by several workers (11 14) The patients of Brigden and Robinson (11) were really heavy drinkers who had consumed large quantities of alcohol daily for more than ten years On the other hand Alexander (3) defines alcoholism in his patients as the daily consumption of at least four pints of beer or two shots of whisky In our material 80 per cent of the patients had been drinking for more than ten years and most of them had consumed greater amounts of alcohol daily than those mentioned by Alexander It might be of importance however that 80 per cent of our alcoholics were periodic drinkers which is the prevailing type of alcoholism in Finland The drinking periods had varied from a few weeks to several months the interims being usually shorter As a rule the patients ate very little during the periods of alcohol consumption gaining their caloric intake mainly from the alcohol Between those periods their diet was normal It remains open to discussion whether this type of periodic alcoholism is less toxic to the heart than continuous alcohol consumption with normal or high caloric intake

Another possible explanation of the absence of alcoholic cardiomyopathy in the present material might be an overall low incidence of cardiomyopathy in chronic alcoholics In an extensive study on heart diseases of unknown origin von Bonsdorff (8) also analyzed autopsy findings of 189 patients with alcoholic liver cirrhosis After exclusion of all cases with a known etiological cause there remained 19 hearts with an increase in fibrous interstitial tissue most probably connected with the patient s alcoholism but only four of these patients had shown signs of congestive heart failure In a similar study (9) of 160 cases of anatomically verified alcoholic cirrhosis of the liver 17 enlarged hearts without any evident etiologic cause were found In four of them there had been clinical signs of heart disease In a recent study from Norway the mortality of chronic alcoholics was evaluated by Sundby (40) He found that death from heart failure was common among alcoholics whose death had been attributed to "chronic alcoholism and was probably due to alcoholic cardiomyopathy However since in this material of 1 061 deaths only 23 were attributed to chronic alcoholism the incidence of possible cardiomyopathy remained as low as 1 to 2 per cent If this number represents the actual frequency of alcoholic cardiomyopathy in Scandinavia it is understandable that not a single case was found in a clinical material of 100 alcoholics

Indeed in clinical studies in which patients have been selected on account of their alcoholism

he prevalence of heart disease is low Frederik sen and Hed (16) studied the ECGs of 121 alcoholics under 41 years of age Although subjective discomfort such as dyspnea and palpitation was common only one case of manifest heart disease with enlargement of the heart is mentioned by the authors Obviously their material consisted of heavy periodic drinkers Suárez and Suarez (39) who studied a series of 52 alcoholics did not find clinical manifestations of alcoholic cardio myopathy Even in the series of Priest et al (35) none of the patients had serious cardiac symp toms or showed evidence of heart failure

The high frequency of alcoholism in patients with unknown heart disease may have several explanations The patients with alcoholic cardiomyopathy probably come from a large popula tion of alcohol consumers On the other hand cardiomyopathy from any cause will readily be classified as alcoholic if the patient happens to consume alcohol There remains also the possibil ity that other substances than alcohol present in alcoholic beverages such as cobalt in beer may cause the local occurrence of toxic heart disease The high percentage of beer drinkers in some of the materials of alcoholic cardiomyopathy (3 11) could point in this direction It is also of interest that 16 of the Quebec beer drinkers who returned to their drinking habits consuming as much beer before but without added cobalt did not show y symptoms or physical signs of cardiovascular disease at a follow up study one year later (34)

Further studies on alcoholics are needed to evaluate the frequency of specific heart disease due to alcohol

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INTESTINAL EXCRETION OF LOW AND HIGH MOLECULAR WEIGHT POLYVINYLPYRROLIDONE (PVP) IN PATIENTS WITH PROTEINURIA

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Abstract Excretion of polyvinylpyrrolidone (PVP) by the gastrointestinal tract has been investigated in a group of patients with proteinuria due to primary renal disease and a group of normal controls Two types of PVP were used one with a mean molecular weight of 40 000 labelled with ^{125}I (LMW–PVP) the other with a mean molecular weight of 160 000 labelled with ^{131}I (HMW–PVP) The purpose of this study was to obtain more information about the possibility of increased gastro intestinal protein loss in patients with proteinuria and, if present, to acquire data on its mechanism. In six of 30 patients PVP excretion was definitely above normal Mean faecal PVP excretion was nearly twice as high as compared with the control group The data obtained in this investigation point to a simultaneous involvement of the capillary wall in the gut in at least some of the patients with the nephrotic syndrome A preferential excretion of the LMW–PVP was shown in both groups which indicates a sieving effect in the excretion of macromolecules Faecal PVP excretion however appears to be less selective than urinary PVP excretion. A theory is developed to explain the gradually decreasing ratio

$$\frac{\text{LMW–PVP excreted in stools}}{\text{HMW–PVP excreted in stools}}$$

in the two groups

Proteinuria is the main cause of hypoproteinaemia in patients with the nephrotic syndrome An increased extravascular volume may also be of importance As a third mechanism increased gastrointestinal protein loss in nephrotics has been under consideration This possibility was initially suggested by Freeman and Matthews (4) who found a discrepancy between the disappearance rate of total body activity as compared to intravascular activity after intravenous injection of ^{131}I albumin in a nephrotic patient More recently studies with ^{131}I PVP or ^{51}Cr albumin showed a higher than normal faecal loss in patients with proteinuria (3 7 8) The number of patients studied so far is relatively small while the groups of patients were also heterogeneous with respect to the cause of the proteinuria *Only in some cases was either* biopsy or autopsy material available for histological studies of the kidneys

So far there seems to be no general agreement about the frequency of gastrointestinal protein loss in nephrotic patients The purpose of this investigation was to obtain more information about this serum protein loss in a group of patients with primary renal disease and when increased about its mechanism The possibility of preferential PVP excretion dependent on molecular size was investigated by using two types of PVP with different molecular weight range

MATERIAL AND METHODS

Thirty patients (14 females and 16 males) with proteinuria due to primary renal disease were studied Ages ranged from 7 to 73 years In all patients a renal biopsy was performed The histological diagnosis, a brief review of the relevant clinical data, some laboratory data and the therapy at the time of study are given in Table I

Simultaneously 27 normal control subjects were examined. They had no evidence of renal or gastrointestinal disease or other conditions, known as a possible cause of gastrointestinal protein loss Control subjects were *matched for age and sex.*

Two types of polyvinylpyrrolidone (PVP) were obtained as pyrogen free sterile isotonic solutions from the Radiochemical Centre Amersham, England. Mean molecular weights were reported as 40 000 and 160 000 respectively The low molecular weight PVP (LMW–PVP) was labelled

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Table I *Clinical data results of some laboratory investigations and excreted amounts of PVP in 30 patients with proteinuria due to primary renal disease stools were collected during four days after PVP infection*

Pat no	Age	Sex	Biopsy diagnosis	B P	Therapy: Sodium poor diet	Therapy: Protein rich diet	Therapy: Diuretics	Therapy: Steroids
1	49	♂	Membr glom nephr	140 90	+	+	−	−
	57	♀	Focal lo al glom nephr	170 90	+	−	−	−
3	30		Membr glom nephr	185/105	+	+	+	−
4	78		Membr glom nephr	135/ 80	−	−	−	−
5	66	♀	Focal local glom nephr	170/ 90	+	+	+	−
6	7		Minimal lesions	170 80		+	−	+
7	18		Prolif glom nephr	150 100	−	+	+	−
8	15	♀	Membr glom nephr	180/100	−	+	+	−
9	39	♀	Membr glom nephr	150/ 90	+	−	+	−
10	4	<	Membr prolif glom nephr	140/ 90	−	+	−	−
11	5		Membr glom nephr	170 95	−	−	−	−
12	12		Focal local glom nephr	145/ 80	+	−	−	+
13	15		Prolif glom nephr	150 105		+	−	−
14	34		Membr glom nephr	130/ 70	−	−	−	−
15	39	♀	Prolif glom nephr	1 0 60	+	−	−	−
16	73		Membr glom nephr	185/ 85	+	−	−	−
17	19		Membr glom nephr	130/ 80	−	+	+	−
18	51		Minimal lesions	140/ 80		−	+	−
19	67	♂	Membr prolif glom nephr	205/115	+	+	+	−
0	37	♂	Minimal lesions	160 105	+	+	+	+
1	49	♀	Membr prolif glom nephr	180 110	−	−	+	−
[illegible]	16	♂	Membr glom nephr	145/ 80	+	+	−	−
3	5	♂	Focal local glom nephr	160 100	−	−	−	−
4	41	♀	Focal local glom nephr	155/105	−	−	−	−
5	45	♂	Membr glom nephr	160 100	+	−	+	−
	31		Membr glom nephr	160/ 90	−	−	−	−
	39	♀	Membr glom nephr	150/ 95	−	−	−	−
	39	♀	Membr glom nephr	165/100		−	−	−
9	3	♀	Membr glom nephr	130 80		−	+	−
30	4		Lobular glom nephr	130 90		+	−	−

with [131]I the high molecular weight PVP (HMW-PVP) with [125]I Analytical ultracentrifuge studies and gel filtration experiments, carried out in the Chemical Laboratories, University of Groningen, showed that the tailing of the HMW-PVP in the low molecular weight range was not important. PVP is delivered with a sachet containing an ion-exchange resin which should maintain free iodine content below 1%. This was confirmed in our laboratory by dialysing experiments.

Twenty patients and 15 normal controls were intravenously injected with .5 μC [125]I-PVP and 75 μC [131]I PVP mixed shortly before injection. Ten patients and 1. normal subjects received only LMW-PVP. Thyroid uptake of free iodine was blocked with Lugol's solution.

Stools were collected during four days after injection. Contamination of stools with urine was prevented carefully. No occult blood loss was detectable.

For determination of serum half life value of both types of PVP blood samples were taken 10 45 150 and 4 0 min after injection and also daily during four days in eight patients and seven control subjects. Disappearance curves were plotted and extrapolated to time zero. Serum half life time that is the time to fall to one half of the T value could then be calculated.

Radioactivity was estimated in a Tobor scintillation detector (Nuclear Chicago) coupled to a pulse height analyser. The photopeaks of [125]I and [131]I (35 KeV and 360 KeV respectively) are located very far apart and the fraction of the Compton spectrum of [131]I seen in the window set for [125]I is small (about 10% of the events counted in the photopeak). Radioactivity was compared with a standard solution and expressed as a percentage of the injected dose.

Proteinuria (expressed as g .4 h) was estimated by the biuret method. The values given in Table I are the mean of four successive daily determinations. Total serum protein level was estimated by the biuret method. Serum albumin was estimated by means of paper-electrophoresis

Serum					Urine			Faecal excretion (of injected dose)		
Serum protein (g*)	Serum albumin (g*)	Serum creatinine (mg°)	Urea (mg°)	Choles terol (mg)	Protein uria (g/24 h)	Amino N (mg kg/ 24 h)	Selective (S) or non selective (N) type of protein uria	LMW–PVP	HMW–PVP	Ratio LMW/HMW–PVP
6 34	4 5	1 4	63	202	1 1	6 5	N	1 61	0 94	1 71
7 24	3 4	1 7	49	241	0 9	1 0	N	0 73	0 54	1 35
5 59	3 3	1 6	45	209	4 3	2 0	N	1 74	1 37	1 27
6 40	4 5	0 9	35	216	0 7	4 0	S	2.62	.24	1 16
6 34	3 7	1 4	25	316	3 5	1 1	N	0 74	0 53	1 40
6 00	8	—	30	427	0 3	—	S	5 22	6 25	0 83
3 33	1 1	2 0	51	525	20 2	7 9	N	1 68	0 73	2 30
5 00	2 8	1 ?	53	295	10 1	7 0	N	1 12	0 95	1 18
6 68	*2 8*	*1 1*	*44*	*238*	*2 9*	*6 5*	*N*	*0 78*	*0 43*	*1 81*
5 87	2 8	0 9	35	217	2 7	3 0	N	0 51	0 40	1 28
6 6	3 2	0 9	36	281	1 4	1 5	N	1 13	0 70	1 57
6 30	? 8	0 7	20	259	0 7	—	S	1 11	0 58	1 86
5 0?	2.5	0 9	47	241	5 8	2 3	N	0 56	0 44	1 27
5 55	3 5	1 2	55	?37	4 4	1 0	N	0 51	0 34	1 50
6 74	3 8	2 4	40	200	3 4	1 0	N	0 43	0 44	0 97
5 95	3 4	1 4	52	233	1 8	0 9	S	0 84	0 78	1 08
4 48	1 8	0 6	23	308	8 6	4 7	N	1 24	1 00	1 24
5 40	3 0	0 5	25	244	1 1	1 5	S	0 59	0 54	1 09
3 81	2.0	1 4	59	300	7 7	3 4	N	1 50	0 96	1 56
3 40	1 3	1 3	60	371	16 3	4 9	S	1 83	0 80	2 28
7 91	5 1	1 3	35	202	0 3	—	N	0 39		
3 74	1 2	0 8	36	343	19 5	—	N	0 53		
7 10	4 4	1 0	39	.14	7	—	N	0 48		
8 19	5 2	0 9	35	290	3 0	—	N	0 45		
6 21	2 5	1 2	46	415	10 8	—	N	0 28		
7 05	4 6	0 8	33	202	1 0	—	N	0 97		
7 19	4 6	0 9	2	275	2 0	—	N	0 68		
4 95	2.8	2 6	65	260	11 3	—	N	1 00		
5 12	2.4	1 2	38	349	10 5	—	N	0 36		
5 01	2 3	1 6	48	298	8 8	—	N	1 10		

with an Analytrol (B ckman Spince) counter Serum con centrations of creatinine and urea were determined with an auto-analyser according to Jaffé and by the diacetyl monoximmethod respectively Serum cholesterol was estimated according to Liebermann–Borchard and serum lipids were determin d gravimetrically by the extraction method of Bloor

RESULTS

The results of patient no 6 are omitted because they are considered to b- inaccurate The high faecal PVP excretion in this child was very probably due to contamination with urine as nearly all activity was found in the first 24 hour stools

In Table I a revi w of some relevant clinical and laboratory data is given The diet is called protein rich when patients had a daily protein intake exceeding 100 g When diuretics were ad ministered *these were generally benzothiadiazine* As can be seen proteinuria varies from 0 3–20 2 g/24 h in this group of patients about one third of the patients with proteinuria had a true ne phrotic syndrome and six patients had a selective type of proteinuria Further data on these subjects and definitions about selectivity will be given in another paper (1)

In Fig 1 faecal LMW–PVP excretion in pa tients and normal controls is plotted In six pa tients LMW–PVP excretion was definitely above normal The difference between the mean values in the patient group (0 94 %) and in the control group (0 54 %) is statistically significant ($p <$ 0 005)

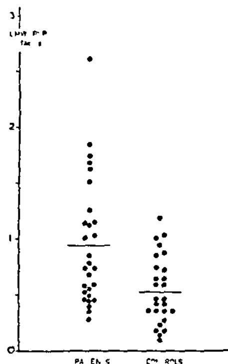


Fig 1 Gastrointestinal excretion of LMW-PVP (expressed as percentage of injected dose) in patients with proteinuria and normal controls The difference between the mean values in both groups is significant (Student's *t* test $p < 0.005$)

Mean faecal HMW-PVP excretion in the group pa ients was 0 77 % in the group of normal jects 0 .2 This difference is also significant ($p < 0.001$)

No correlation existed between LMW-PVP excretion in the stools and serum albumin level Nor could a correlation be found between faecal LMW-PVP excretion on the one hand and the

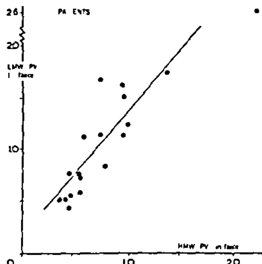


Fig 2 Correlation between amounts of LMW-PVP and HMW-PVP excreted in stools in patients with proteinuria (Spearman $p < 0.001$ $y = 1.2x + 0.2$)

degree of proteinuria or total serum lipids on the other The same holds true for HMW-PVP

Table II shows the serum half life of both types of PVP in eight patients and seven normal controls Mean half life of both types of PVP is a little shorter in the control group as compared with the patient group the differences are however not significant From Table II it is clear that the half life of LMW-PVP was shorter than the half life of HMW-PVP in all patients and controls

In the group of patients and in the group of controls no correlation could be found between the excreted amounts of LMW-PVP in the stools and the serum half life The same holds true for HMW-PVP

Table II *Half life in serum of LMW-PVP and HMW-PVP in eight patients with primary renal disease and seven control subjects*

Pat. no	Half life in serum (h)		Control subject no	Half life in serum (h)	
	LMW PVP	HMW PVP		LMW-PVP	HMW PVP
8	14	17 5	1	12	17
13	17	17 5	2	17	19 5
14	8 5	9 5	3	8	9
15	13	17 5	4	9	17
16	14 5	18	5	13 5	15 5
17	12	15	6	9	10 5
18	14 5	18 5	7	9	16
19	11	14			
Mean	13	15 7		11 1	14 9

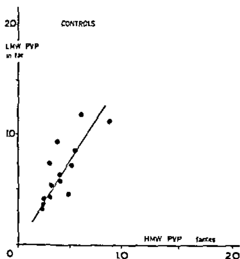


Fig 3 Correlation between amounts of LMW–PVP and HMW–PVP excreted in stools in normal controls (Spearman $p < 0.01$ $y = 1.4x$)

From Table I it is clear that in 18 of 19 patients with proteinuria the ratio LMW–PVP excreted in stools/HMW–PVP excreted in stools was higher than 1 0 Statistically this ratio differs significantly from 1 0 ($p < 0.001$) In the group of control subjects 13 of 15 appeared to have a ratio higher than 1 0 This ratio also differs significantly from 1 0 ($p < 0.01$) This means that in both groups faecal excretion of LMW–PVP exceeds HMW–PVP excretion

Fig 2 demonstrates the positive correlation between the amounts of LMW–PVP and HMW–PVP in the stools in the patient group ($p < 0.001$) In the control group (Fig 3) the same holds true ($p < 0.01$) Regression lines were calculated in both groups but no significant difference was found between the slopes of the two lines Equally no significant difference was found between the mean ratios of the patient and control groups (respectively 1 5 and 1 4)

Nine patients and eight normal control subjects had a daily bowel movement during the observation period Mean ratios on the four successive days after intravenous PVP injection for these patient and control groups were computed As can be seen in Fig. 4 the ratio gradually decreases with time This was statistically significant ($p < 0.02$)

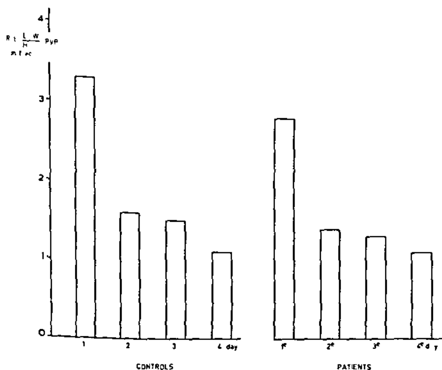


Fig 4 Mean ratio LMW-PVP HMW-PVP in stools on four successive days after intravenous injection in nine patients and eight control subjects. Ratio decreases significantly (Kruskal & Wallis $p < 0.0?$)

DISCUSSION

From the literature only scarce information is available about gastro-intestinal protein loss in patients with a nephrotic syndrome In the reported studies (3 7 8) patients were included with pyelonephritis amyloidosis diabetic nephropathy and severe azotaemia Patients with a nephrotic syndrome due to amyloidosis may lose serum proteins in the stools as a consequence of amyloid deposition in the gut and patients with azotaemia as a result of uraemic enterocolitis Therefore in this investigation a group of 30 patients was selected with proteinuria due to primary renal disease without evidence of diseases or known complications in the gastrointestinal tract No patient had significant renal insufficiency As mentioned above the results in patient no 6 will not be discussed

In earlier studies (5) we gave to over 60 normal controls the same type LMW–PVP though from different batches The highest four day excretion in the stools was less than 1 2 % of the injected amount So it seems justified to consider an excretion of 1 4 % or more to be abnormal This reflects a raised gastrointestinal protein loss (6) From this investigation it is clear that six patients out of 29 had an abnormal LMW–PVP excretion in the stools

The faecal excretion of PVP did not correlate h the kind of therapy In the cases in which roid treatment was given no correlation with the duration of therapy was found Our observation differs in this respect from that of Kluthe et al (8) who found a normal excretion of PVP in patients who were on steroid treatment over a period of more than three months Further data appear to be necessary to evaluate the influence of steroids on protein loss in the gut in patients with proteinuria

In the patients with an abnormal PVP excretion two had considerable oedema one had slight and three had no oedema In the patients with a normal PVP excretion three had considerable four had little and six had no oedema No patient had abdominal complaints except one who had a bowel movement three to five times a day He excreted a normal amount of PVP Therefore on clinical grounds the patients with raised PVP excretion could not be distinguished from those with a normal excretion

There was no correlation between faecal activity and the results of a number of blood and serum investigations (Table I) Examination of the stools revealed no signs of an impaired digestion or steatorrhoea as seen occasionally in patients with proteinuria and gastrointestinal protein loss (7) Nor was a correlation found between the excreted amounts of PVP and serum cholesterol levels in contrast with the findings of Bennhold and Ott (3) Their observation must be attributed to a coincidence in the very small number of patients

The following have to be considered as possible mechanisms for the increased gastrointestinal protein loss in patients with proteinuria due to primary renal disease

1 Decreased reabsorption of radioactivity from the gastrointestinal tract in the patient group
2 Oedema of the gastrointestinal wall as part of generalised hypoproteinaemic oedema resulting in leakage of oedematous fluid
3 Prolonged elevated blood levels of the injected testing substance as a consequence of decreased urinary excretion In this case the finding of increased amounts of PVP in the stools would not imply that there exists an increased protein loss in the gut
4 Increased permeability of the capillary wall in the gastrointestinal tract mucosa

Data from the literature (7) and our investigation do not point to a decreased reabsorption in the gastrointestinal tract in patients with proteinuria Therefore the possibility mentioned under 1 seems unlikely

It seems improbable that the increased gastrointestinal protein loss is caused by oedema of the gut Only seldom in intestinal biopsy specimens are distinct signs of oedema found even in the presence of peripheral oedema (7) Furthermore in X ray examinations of the bowel symptoms of oedema were found to be slight and inconsistent (7) In addition in our series no correlation was found between the degree of proteinuria or serum albumin concentration and the excreted amounts of PVP This appears to be a strong argument against the theory mentioned above as the degree of hypoalbuminaemia is one of the best parameters for the appearance of hypoproteinaemic oedema In fact in several patients with a normal protein level and without oedema an abnormal PVP excretion was found.

From the work of our group which will be published in another paper (1) it is clear that urinary excretion of LMW–PVP and HMW–PVP in patients with proteinuria is reduced compared with normal controls However serum half life of the labelled PVP in the patient group was only a little longer than the half life in the group of normal controls Moreover there was not even the slightest indication of a positive correlation between serum half life of the two types of PVP and the amounts excreted in stools of patients or controls Therefore we believe that the sometimes considerably increased PVP excretion in the stools is not due to the slightly increased half life in serum

It must be concluded that increased faecal PVP excretion in a number of patients with proteinuria due to primary renal disease indicates a true increased serum protein loss Hence we believe that the evidence points to a simultaneous involvement of the capillary wall in the gut as a concomitant expression of the renal disease

Immunologic investigations have shown that many plasma proteins are demonstrable in jejunal or ileal fluid including proteins with high molecular weight (2 5) It was therefore stated that there would be no molecular sieving effect in the gastrointestinal tract This is in contrast with the excretion pattern of proteins in the urine of patients with a nephrotic syndrome in which protein clearances at least for the most part are dependent on molecular weight

The capillaries of the glomeruli and capillaries of the villous layer of the gut resemble each other anatomically (9) Hence an absolute absence of any influence of mo ecular weight on protein excretion in the gastrointestinal tract is improbable The difficulties in obtaining adequate non contaminated intestinal fluid in which protein digestion is sufficiently prevented are numerous (5) Determinations of plasma/jejunal fluid protein clearances therefore seem to be unreliable We believe that an inert macromolecule is preferable in investigations concerning the capillary transfer mechanism It also permits comparison of the excretion pattern in the gut and in the kidney The use of labelled LMW–PVP and HMW–PVP is suitable for this purpose (1)

The results of this study show that there is a preferential excretion of LMW–PVP in normal subjects and in patients with proteinuria This means that to a certain extent there is a sieving effect of capillaries in the gastrointestinal tract When the excreted amounts of both types of PVP in stools are compared with those in urine (1) it is however clear that gastrointestinal excretion of macromolecules is less selective than renal excretion for in every patient and normal control the ratio LMW–PVP HMW–PVP in urine exceeds the ratio in stools

The amount of excreted PVP varies individually but if a subject s LMW–PVP excretion is relatively high then the excreted HMW–PVP is proportionally increased This is expressed in the regression lines (Figs 3 4) No statistically significant difference could be shown between the slopes of these lines in the two groups studied This points to the fact that permeability has not changed qualitatively in the patient group This is also expressed in the practically equal mean ratios in patient and control groups respectively 1 5 and 1 4

The gradually decreasing ratio (Fig 4) is a remarkable finding There are four possible explanations for this phenomenon

1 Firstly it is possible that the smaller molecules enter the lumen of the gut faster than molecules with a high molecular weight The same excretion pattern is found in urine (1)

2 The stools from the first 24 h consist mainly of material present in the distal parts of the gastrointestinal tract at the time of injection Therefore it is possible that the high ratio in the first portion of faeces is caused by a relatively smaller permeability for HMW–PVP in the distal parts of the gut compared with the proximal parts

3 It is well known that a small percentage of PVP is reabsorbed especially free iodine and small molecules (3 6) As stools from the successive days remained longer in the lumen of the gut, a relatively greater amount of LMW–PVP will have been absorbed This also will result in a decreasing ratio

4 The preferential excretion of LMW–PVP in urine and stools causes a prolonged higher blood level of HMW–PVP this is reflected by a longer serum half life of HMW–PVP as compared with LMW–PVP Compared with the first day the capillary wall will be presented with a relatively higher amount of HMW–PVP on the following days For this reason the ratio decreases also

Further investigations which it is hoped will give more information on this decreasing ratio are in progress

ACKNOWLEDGEMENT

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INTERMITTENT INTRAHEPATIC CHOLESTASIS OF UNKNOWN ETIOLOGY IN FIVE YOUNG MALES FROM THE FAROE ISLANDS[1]

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Abstract Clinical and biochemical findings in five patients from the Faroe Islands with intermittent intrahepatic cholestasis of unknown etiology are described This brings the number of recorded cases which fulfil the suggested criteria for the syndrome up to 24 The Faroe patients are all males and born between 1938 and 1943 In three patients the episodes of jaundice started during the first years of life in two they started after puberty Four of the patients had symptoms of pancreatic affection, in one the diagnosis of chronic pancreatitis was confirmed by calcifications demonstrated on X ray Two patients are distantly related, and one patient has a sister who presumably suffers from the same disease It is conjectured that the pathogenesis is a defect in bile acid metabolism

The syndrome of intermittent intrahepatic cholestasis was first described in two patients by Summerskill and Walshe in 1959 (24)

The syndrome is characterized by 1) several episodes of pronounced jaundice with severe pruritus and biochemical signs of cholestasis 2) bile plugs in the liver biopsy 3) normal intra and extrahepatic bile ducts on direct cholangiography 4) absence of factors known to produce intrahepatic cholestasis occasionally as drug intake or pregnancy and 5) symptom free intervals of several months or years These characteristics may be used for a definition of the syndrome if arbitrarily at least three episodes of jaundice and a free interval of at least six months are required This will separate the syndrome from protracted and relapsing hepatitis

[1] This work was in part presented at the International Symposium on Jaundice Freiburg i B Germany October 1967

Twenty four patients with a case history concordant with this definition have been reported (1 2 8 9 10 14 16 17 22 23 24 25 26 29) including the present series of five patients Two patients from this series (cases 1 and 2) were presented in 1960 (26)

CASE REPORTS

Case 1

Born 1942 At the age of 2 and 4 years jaundice and pruritus lasting for several months, otherwise in good health until the age of 12 when a new episode occurred starting with sharp abdominal pain. Laparotomy in January 1955 showed a slightly enlarged, dark, but otherwise normal looking liver and normal extrahepatic bile ducts. No biopsy or cholangiogram was made The pancreas was described as harder than normal. A cholecystogastrostomy was performed. The following episode of jaundice started when he was 15 years old he was transferred to Rigshospitalet, Copenhagen, where laparotomy in February 1958 showed slightly enlarged liver slender bile ducts (peroperative cholangiography) normal spleen and pancreas. The gall bladder was removed. Since then repeated attacks (Fig. 1) but well-being between the attacks

Case 2

Born 1943 First attack at 9 months for the following 7 years periods of jaundice of 2 to 3 months duration once to twice a year Laparotomy in January 1950 showed macroscopically normal liver and the bile ducts appeared normal. No biopsy or cholangiogram was made No attacks from the age of 7 to 15 years since then regularly jaundiced (Fig. 1) During the free intervals no complaints except for food allergy

Case 3

Born 1938 From the age of 1 to 6 years regularly jaundiced starting each December and lasting for

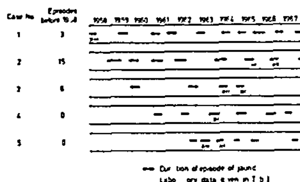


Fig 1 Episodes of jaundice during ten years.

6 months At the a e of ?? years, whi e fishing in Greenland waters, a new episode of jaundice starting with slight arthralgia Laparotomy in March 1960 showed a dark but otherwise normal liver normal extrahepatic bile ducts No biopsy or cholangiogram Since then three episodes with a typical course except for slight arthralgia

Case 4

Born 194 No jaundice or other significant disease during infancy and childhood the first episode of jaundice occurred at the a e of 19 years Laparotomy in March 1961 showed normal peroperative cholangiogram macroscopically normal liver and pancreas. Since then five similar ep sodes and perfect we l being during the interval

Case 5

Born 1941 Normal development during infancy and childhood The first episode of jaundice occurred at the a e of 21 years, while he was fishing in Greenland waters. Laparotomy in January 1963 showed normal bile duc s at cholangiography the liver looked normal (biopsy was taken) and the pancreas was felt to be a litt e harder than normal During the following 3 years four more episodes of jaundice with a similar course Another laparotomy was performed in October 1965 some concrement could be felt in the gall bladder but as it was without inflammatory changes, and choledochotomy showed a normal common duct cholecy tectomy was not performed Du to recurrent attacks of sharp abdominal pain and high u ine amylase a third laparotomy was perf rmed at R gshospitalet in June 1966 The liver appeared normal the otherwise normal gall bladder contained eight sma l dark concrements the ductus choledochus was s ghtly d lated and the cholangiogram revealed a stenosis ju t below the entrance of the normal cystic duct, possibly caused by the previous choledochotomy The stenosi wa resected The head of the pancreas was indurated and nodular a in chronic inflammation no biopsy of the pancreas was taken. Five days postoperatively cho ascos de loped and a leakage at the site of resection had to be repa red The patient was well until one year after the operation since then he has had three episodes of relatively mild jaundice and moderately seve e abdominal pain

Family histories

None of the patients were closely related but grea grandfathers of cases 1 and 2 were brothers

The occurrence of similar symptoms among the rela tives of the patients was only noted in one case The sister of cas 3 born in 194 was severely jaund ced for several months when she was 4 years o d She had been complaining of abdominal pain and itching for some time before She was treated by bed rest at home and no tests were made At the a e of 17 she suffered from general malaise itching and periodically pale stools for about 6 months, followed by intense jaundice pruritus and abdominal discomfort for about 2 months. The icteric index was 108 alkaline phosphatases 25 KA units, and alanine transaminases were normal No liver biopsy or surgery was performed the jaundice and pruritus disappeared rather rapidly and a cholecystogram shortly afterwards was normal Since then she has had two normal pregnancies without jaundice or pruritus She has not taken contraceptive pills

The mother of case 5 has been jaundiced twice once with prolonged s vere itching Jaundice of pregnancy among the relatives of the patients has not been recorded

Clinical findings during episodes of jaundice

The pa ients were not aware of precipitating factors As seen in Fig 1 the episodes in some cases occurred with some regularity but no fixed seasonal pattern can be recognized The feeding habits of the patients were unremarkable and they d d not take drugs of any kind An episode of jaundice usually starts with fatigue loss of appetite nausea and sometimes vomiting Simultaneously or a few days later itching starts disturbing the sleep at night Dark urine and pale stools follow shortly afterwards and then jaundice becomes apparent first scleral then universal Constant pain centrally in the abdomen between the xiphoid process and the umbilicus without irradiation often occurs during the first week of an at ta k and this is occasiona ly the initial symptom In cas 5 the pain usually is intens requiring repeated injections of strong analgetics and in case 2 it is mild or absent In the remaining patients the pa n is moderate

The fatigue and poor appetite persist during the episode causing a weight loss of s veral kilograms Usually the first sign of rem ssion is the return of the appetite then some color appears in the stools the itching diminishes and often disappears while the jaundice is sti l quite marked

During the free intervals the patients appear clinically healthy and have no specific complaints, but still they may have difficulties in resuming their normal ac i ies. because they fear new attacks Most young men at the is ands are fishermen being at sea for several mon hs at a time All the pat ents had been out fishin for a period but were unable to continue this work

It i uncertain whether aborti e episodes occur Fre quently the patients have complained of periods wi h some fatigue slight itching, and transitory changes in the color of the urine and stools but without confirmation by laboratory tests The complaints may be ascribed to an understandable anxiety regarding the possib l ty of the approach of a new episode of jaund e

Laboratory findings

The result of some selected tests at different stages are given in Table I

The course of the serum bilirubin (12) alkaline phosphatases (13) and alanine transaminases (15) in a typical case is shown in Fig. 2 The bilirubin curve in most cases is relatively smooth The maximum bilirubin value and the slope of the curve during recovery varies considerably from one episode to another in the same patient The level of serum alkaline phosphatases generally follows the bilirubin curve but becomes normal much later The serum alanine transaminases are rarely excessively elevated. The highest values are constantly observed during the recovery period often a biphasic course is found with the lowest values during the culmination of the episode

During the initial phases high concentrations of prothrombin-proconvertin have been found during prolonged attacks the values may become abnormally low but they always react promptly to treatment with vitamin K. The serum albumin (paper electrophoresis) sometimes falls to subnormal values and becomes normal during the recovery period the γ-globulin showed a transient rise in case 5 during a severe attack but otherwise it remains within normal limits. The α- and β globulins are regularly elevated to about twice the upper normal limit and fall to normal levels during recovery

Serum cholesterol (19) is usually elevated during the culmination of the episode and returns to normal or low values during the recovery This is to a large extent due to changes in nonesterified cholesterol which may be lower than normal during recovery

The BSP and storage (28) and the galactose elimination capacity (7) were determined during symptom free intervals in four patients (Table II) In case 1 the tests were performed just prior to and in case 2 shortly after an attack, and thus may not represent truly basal" values The BSP was reduced in all whereas BSP storage and galactose elimination were below the normal limit only in case 1

Liver biopsies were performed on the dates given in Table I and also during some of the laparotomies as mentioned in the case reports During the episodes of jaundice numerous bile plugs are found in the bile canaliculi together with signs of moderate liver cell damage such as a few necroses, some multinucleated liver cells, and variable stainability of the cytoplasm The portal tracts show slight to moderate inflammatory infiltration mostly with mononucleated cells, but also a few neutrophil and eosinophil granulocytes Biopsies taken during the free intervals are essentially normal A detailed description of the light and electron microscopic picture will be given elsewhere

The gall bladder and bile ducts cannot be visualized by cholecystography during the episodes but they appear normal during the intervals except for the calculi found in case 5 The increase in urine amylase usually correlates well with the abdominal pain In case 5 pancreatic involvement was also demonstrated radiologically by retroperitoneal calcifications Pancreas secretion studies, performed in case 1 (1965) and case 5 (1967) did not reveal decreased pancreatic function

The ECGs were strikingly similar in all the patients showing a negative T wave in lead III There were otherwise no symptoms or signs of heart disease

Therapy

Treatment with adrenocortical steroid and cholestyramine was tried in all patients, but due to the great spontaneous fluctuations of the disease and the impracticability of a controlled trial it is difficult to evaluate the effect Neither treatment could reproducibly suppress or prevent the episodes It is the impression of the patients however that steroids are without effect but that cholestyramine relieves the pruritus and possibly shortens the duration of the jaundice

DISCUSSION

The first one or two episodes of intermittent intrahepatic cholestasis almost inevitably will be misdiagnosed as extrahepatic biliary obstruction and lead to laparotomy When the bile ducts are found to be normal and several similar episodes supervene few diagnostic possibilities other than intermittent intrahepatic cholestasis will exist The clinical picture of intermittent intrahepatic cholestasis is so characteristic and the diagnostic criteria so tangible that it is surprising that the syndrome has not been described earlier This indicates that the syndrome is very rare or less likely has come into existence recently

During the last nine years 24 patients fulfilling the diagnostic criteria mentioned have been reported 18 males and six females Six further cases five males and one female should probably be included viz case 2 of de Silva et al (8) who only had two episodes of jaundice three relatives of the case of Biempica (2 personal communication from dr Arias) one relative of Goldberg s cases (10) and the sister of case 3 in this series

The term intrahepatic cholestasis implies obstruction to the flow of bile within the liver It is used when clinical and biochemical data are interpreted as biliary obstruction and no obstruction is found macroscopically The lesion may be localized to the wall of the bile capillaries i e in the liver cell itself or to the intra and interlobular bile ducts The usual morphological examinations have not revealed the site of the lesion in intermittent intrahepatic cholestasis but by 3-dimensional reconstruction of an interlobular bile duct in the case described by Levy et al (16) obstruction by swollen cells protruding into the lumen of the duct was found

Table I *Laboratory data*

Case no	Date	Serum bilirubin (mg/100 ml)	Serum alanine transaminase (units/ml)	Serum alkaline phosphatase (K-A units)	Serum cholesterol (mg/100 ml)	Serum cholesterol non-esterified (mg/100 ml)	Serum albumin (g/100 ml)	Serum β globulin (g/100 ml)	Urine amylase (units)	Fecal fat (g/24 h)	Bromsulfalein retention (%)	Comments
	Normal	1 0	1 7	10	150 250	31 62	4 44	< 0 79	128	7	5	
1	9 1 58	34 8		—	0[illegible]	105	3 06	1 45	32	—	—	Surgery (17 [illegible]) biopsy I
	25 58	15 [illegible]		—	—	—	—	—	—	48	—	
	8 3 58	7		—	—	—		—	—	—	—	
	5 4 59	1 [illegible]	—	—	—	—		—	—	—	—	
	8 5 58	0 3	—	—	—	—	—	—	—	—	—	
	13 1 66	34 9	[illegible]6	69	272	145	4 21	0 94	64	—	—	Biopsy II (22 1)
	27 1 66	13 2	1[illegible]	81	218	118	3 73	1 11	1024	108	—	
	16 [illegible] 66	6 0	1 8	31	270	52	4 02	0 85	178	—	—	
	22 8 67	1 7	2 5	4	216	5[illegible]	4 83	0 75	256	1	26	Biopsy III (22 8)
2	4 2 59	14 9	11 0	44	—	—	3 54	1 16	—	14	—	Prednisone from 24 3
	13 4 59	11 5	10 2	38	355	100	3 36	1 46	3[illegible]	—	18	
	16 10 64	1 1	4 2	11	184	41	4 01	0 88	—	1	6	Biopsy I (16 10)
	5 7 65	17 4	5 5	16	211	56	4 04	1 04	—	72	—	Biopsy II (5 7)
	17 8 65	10 4	2 8	14	—	—	3 78	0 93	32	54	—	Cholestyramine from 5 7
	14 9 65	5 0	14 0	14	132	37	4 14	0 92	—	—	—	
	21 10 65	1 1	8 7	22	174	25	4 32	0 80	—	10	1	
	17 8 66	10 7	4 3	70	—	—	4 [illegible]8	1 05	16	59	—	Cholestyramine from 15 7
	13 9 66	7 8	3 2	16	—	—	—	—	—	—	—	
	18 10 66	2 4	6 9	24	169	51	4 47	1 14	—	32	—	
	4 11 66	1 2	6 8	24	—	—	—	—	—	—	—	
3	12 7 60	1 1	1 2	17	158	39	4 60	0 55	32	2	6	Biopsy I (12 7)
	15 4 64	7 2	6 3	58	197	29	4 28	0 96	256	6	31	Biopsy II (13 4)
	8 5 64	8 3	1 7	30	1[illegible]4	40	3 77	0 84	178	—	14	
	1[illegible] 6 64	7 1	6 2	8	168	44	4 74	1 02	—	—	—	Cholestyramine from 13 5
	17 7 64	1 0	1 4	19	145	4	5 52	0 66	—	1	4	Biopsy III (21 7)
	10 2 65	7 1	18 0	75	256	59	4 71	1 00	128	—	—	
	9 3 65	5 3	4 3	74	156	23	4 71	0 87	256	—	6	Cholestyramine from 10 3
	3 3 65	9	13 0	48	175	33	4 53	0 89	32	—	—	
	0 4 65	0 8	0 7	2	154	0	4 93	0 60	32	—	—	
4	12 11 63	16 3	7 4	49	274	97	4 27	1 07	3 0	24	—	Prednisone from Nov 63
	6 11 63	13 0	2 1	38	277	100	4 [illegible]4	1 03	1 8	—	—	Biopsy II (21 11)
	10 12 63	6 7	13 0	31	[illegible]6	34	3 19	0 94	—	—	—	
	23 1 64	5	7 7	30	255	46	3 74	1 00	16	—	—	
	11 8 67	0 4	1 5	11	199	56	4 88	0 52	256	4	10	Biopsy III (11 8)
									13			

9	16 4 63	14 4	2	8	1 ?	121	3 82	1 06	>10 4	38	—	I In on f om 24 4
	15 5 63	21 2	5	51	25	100	3 4	1 01	128	—	—	Biop y II (19 6)
	6 6 63	4 8	22 0	55	63	67	4 13	0 94	—	3	—	
	5 7 63	2 1	1 0	40	—	—	—	—	16	—	6	
	8 63	0	3 8	44	188	55	3 94	1 01	32	—	—	Predn one u f 0 7
	12 2 64	15 9	3 0	51	50	145	3 94	0 91	3	15	—	Cholesty am e from 20 2
	11 3 64	4 1	4 5	27	03	23	4 10	0 76	—	5	—	Chole tyramine unt l 13 3
	0 4 64	0 4	2 4	22	144	13	4 25	0 61	—	—	3	Biopsy III (14 4)
	6 10 64	2 4	8	25	—	—	4 98	0 74	—	—	—	
	13 11 64	14 8	1 7	—	—	—	4 15	0 93	>1024	10	—	
	21 12 64	2 3	15 0	29	—	—	4 00	0 95	16	—	—	Cholestyramine from 14 11
	18 1 65	0 7	2 0	18	—	—	4 40	0 73	—	—	—	
	2 6 66	1 9	6 2	17	—	—	4 77	0 55	1 8	—	6	Biopsy V (22 6)

The etiologic factor probably should be sought among the following categories infection toxic agents and metabolic factors Due to the unequivocal signs of liver damage during the episodes several authors (9 29) favor the notion that it is a type of virus hepatitis The great number of relapses in most of the patients however and the apparently complete restitution in the int r vals differ distinctly from the usual course of viral hepatitis and there is no positive epidemiologic evidence to support the hypothesis The liver cell damage can equally well be due to the effect of a toxin or to a metabolic derangement or even to cholestasis as such (21)

An exogenic toxin as the etiologic agent could explain why several cases such as those in the present series occur in small groups In that case the toxin should be wid ly distributed however because the reported cases come from many parts of the world viz from the Faroe Islands five from Great Britain (22 29) four from the United States (1 14 23 24) two from Belgium (9) two from Germany (17) two from Italy (10) one from France (16) one from Japan (8) one from Greece (2) and one from Australia (25) Some episodes in patients from the present series have started during a stay in Denmark Dietary and other environmental toxic agents therefore are less likely An allergic reaction has been suspected in some patients who had other allergic manifestations e g case 2 of the present series and case 2 of Kuhn (14) but no relation between these manifestations and the episodes of jaundice has been demonstrated and in the majority of patients no symptoms or signs of allergy were found In one patient (23) chlorpromazine and norethandrolone did not provoke jaundice and in the present series chlorpromazine has been used as a sedative on several occasions without ill-effects

Thus by exclusion a metabolic defect may be incriminated It is not certain whether the syndrome is hereditary Among the 24 unquestionable cases six occur in three families (10 17 26) and six other probable cases are relatives of certain cases (2 8 10 present series)

The relatively high incidence of the syndrome in the Faroe Islands (five patients among 40 000 inhabitants) conforms with the hypothesis of a hereditary defect since this community tively isolated with a high

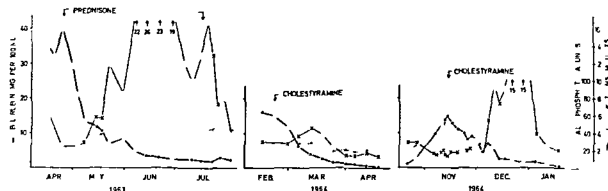


Fig 7 Laboratory data during three consecutive episodes in case 5

marriage It has been suggested that the mode of inheritance is by way of a sex linked recessive (10) but the observations are too incomplete to confirm this The pedigrees of the probands in the present series have been followed as far as possible without revealing further cases or further links between the families of known cases In general the families are large on the islands and the contemporary members know each other well but in most cases only two or three generations can be traced back It must be concluded that if the syndrome is hereditary the dominance of the defect must be very weak

The hepatic uptake and conjugation of bilirubin is considered to be normal (29) Direct reacting bilirubin was found to contribute from less than 50 (7 8 17 24) to about 80 % (2) of total bilirubin In the patients of the present series unconjugated bilirubin was normal or lower than normal in cases 4 and 5 the disappearance of intravenously injected unconjugated bilirubin was greater than normal and in case 5 a considerable amount of alkali stable monoglucuronide was demonstrated (4) Excess formation of abnormal conjugates was suggested to be the metabolic defect of the syndrome but too little is known about these factors in other types of cholestasis to warrant any conclusions

Elevated bile acid concentrations in the serum have been demonstrated in several patients (2 10 24) and are presumeably the cause of the severe pruritus in all the cases (18) but detailed fractionations especially with regard to the concentration of unconjugated lithocholic acid have not been performed Since this acid is capable of producing intrahepatic cholestasis and liver cell damage (25) an abnormal formation or reabsorption of lithocholic acid as the primary metabolic defect in the syndrome must be considered This would also provide an explanation of the intermittence of the jaundice as the cholestasis might prevent the production of lithocholic acid in the intestines for a period The formation of gall stones in case 5 may also be related to abnormal bile acid metabolism (3)

Clinically there are many similarities between intrahepatic cholestasis of pregnancy and the present syndrome Among the six female patients

Table II *Quantitative liver tests during free intervals*

Case no	Normal Date	BSP Tmax (mg/min)	BSP storage (mg/mg)	Galactose elimination (mg/min)	Serum bilirubin (mg/100 ml)
		5–15	20–120	350–700	< 1 0
1	26 8 67	1 3	8	329	1 7
2	2 11 66	3 8	50	368	1 2
4	11 8 67	1 7	49	518	0 4
5	1 9 66	2 0	45	409	0 4

Own determinations.

with intermittent intrahepatic cholestasis the relation between pregnancy and episodes of jaundice has not been marked since coincidence only occurred once in each of two patients (1 23) In one patient an episode started late in a pregnancy and culminated several months after its termination (25) Intrahepatic cholestasis of pregnancy typically recurs during all pregnancies disappears rapidly after the delivery (11) and never occurs outside pregnancy except in some patients when treated with contraceptive pills (20) It is felt that the evidence of a common etiology in both syndromes is meager

The role in the syndrome of the pancreatitis found in case 5 is uncertain Three more patients in the present series had elevated urine amylase and it is likely that the abdominal pain during the initial phases of the episodes of jaundice is due to affection of the pancreas Similar abdominal pain has been described in other cases (10 14 23) but generally in a milder form and without other evidence of pancreatitis The factor which causes the cholestasis (lithocholic acid?) might also affect the pancreas in most cases however to a minor degree

In some instances prednisone (29) and cholestyramine (10 24) appear to have produced a rather dramatic improvement The intermittence of the symptoms makes the evaluation of therapy difficult, but the general impression is that these drugs are of limited value in most of the patients If abnormal bile acid metabolism plays an etiologic role however the effect of bile acid sequestering therapy (6) must be further explored

No cases have been followed for a lifetime and the final outcome is unknown It appears however that the jaundice may continue to recur even if the severity of the episodes and the duration of the free intervals may vary It is also the impression that the recovery during the free intervals is complete but progression to biliary cirrhosis has been suspected in one case (17) and it may be significant that the BSP storage and the galactose elimination capacity in case 1 and the $BSP_{Tm\,x}$ of all the patients examined in this series during free intervals (cases 1 2 4 and 5) were reduced The extensive histochemical and electron microscopic examinations made during a free interval by Biempica et al (2) revealed only minor residual changes but in view of the rather substantial signs of liver cell damage during the jaundice the risk of progressive changes cannot be ignored When the prognosis is evaluated it must also be taken into consideration that the patients mostly are unable to do any work during the episodes on account of general symptoms and will be more or less incapacitated in the intervals as well unless they are very long in part perhaps due to the uncertainty which the constant threat of recurrent attacks imposes It is therefore questionable whether the word benign should be included in the designation of the syndrome (22) From a taxonomic point of view this is superfluous since there is no known malignant counterpart from which the syndrome must be distinguished

Despite the clinical similarities the etiology and pathogenesis need not be the same in all patients with the syndrome Until this is clarified it seems preferable to use a purely descriptive designation The term intermittent intrahepatic cholestasis is suggested because it contains the minimum and adequate information required to separate the syndrome from other types of jaundice and because it can be directly translated into Latin which is used for official diagnoses in many countries

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CORONARY ARTERIOGRAPHY IN VALVULAR HEART DISEASE

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Abstract The authors have studied the preoperative coronary arterioerams in 60 operated cases of aortic val e disease and in 58 cases of mitral valve disease of hom two-thirds ha e been operated on Significant coronary artery chan es were fcund only in two cas s both w th aortic valvular disease Angina pectoris was present in 33% of cases with aortic valve disease most common in aortic stenosis, and in 16 of cases with mitral valve d sease The results show that in valvular heart disease angina pectoris only rarely is due to coronary artery disease This holds at least for the a e groups below 60 years of age to which the majority of the cases in this series belon ed

Angina pectoris and other less well defined types of chest pain may occur in rheumatic heart dis ase This is wellknown in aortic stenosis in whi h angina pectoris occurs frequently The published figures vary considerably depending on the selection of cases Chest pains are also com mon in aortic regurgitation and not rare in mitral valv disease especially in cases with pulmonary hypertension

There are different opinions on the etiology of angina pectoris in valvular heart disease From prognostic and therapeutic points of view it is es ntial to diff r ntiate between pain due to coronary heart disease and to the valvular defect per se It is the aim of this r port to present our findings on coronary arteriograms in cases with aorti and mitral valve diseas

MATERIAL

From a series of cases with severe aortic valve disease we have selected 60 whose records were available and for whom coronary arte iograms of good quality were obtained preoperatively All cases had been operated on, and in nearly all a ball valve prosthesis was inserted The d agnoses and age groups are presented in Table I There ere 46 men and 14 women Cases with aortic stenosis had a peak systolic gradient between 50 and 140 mm H in four cases 100 mm Hg or more Cases with aortic regurgitation had a grade 4 re urgitation (on a scale 1–4) a cordin to thoracic aortoeraphy (7) Cases with stenos s and re urgitation had a pressure gradient between 15 and 170 mm Hg and a regurgitation of grade 3 or 4 In s ven cases aortic valve disease was combined with mitral ste nosis (2 cases) ventricular septum defect (4 cases) or aortic aneurysm (1 cas)

From our files we have randomly s ected 58 cases with mitral valve d sease 36 of which have been operated on The non-operated cases are either on the waiting list for operation ha e decli ed operation or ha e been considered too ad anced for major heart surgery There *were 22 men and 36 women The d agnoses and the age* distribut on are seen in Tab e I

METHODS

From the case histories the occurrence of chest pain was noted and c assified as typical angina pectoris or of other types called atypical angina In cases with typical angina p ctoris, the localization and irradiation of pain, its rela tion to physical work as well as its time relationship were those of angina pectoris (2)

The coronary arteriograms were performed preopera ti ely Details of our technique ha e b en reported earl er (4) We have used a non-s ective method, with contrast injection in the root of the aorta, with the pat ent in the left anterior ob iqu position Bip anar full size simul taneous exposures were used in 107 cases (Fi 1) and cineradiographic recording in one or two planes in 11 cases The findin s were classified from grade 0 to grad 4 s e Table II

RESULTS

The results are summarized in Table III Chest pain classified as typical angina pectoris was noted in 33% of cases with aortic valve disease It was more common in pure aortic stenosis (40%) and in stenosis with regurgitation (53 %) than in pure aortic regurgitation (21%) and was seen only once in the group of aortic valve diseas

Table I *Distribution with respect to diagnosis and age*

Diagnosis	Age group years 16–29	30–49	50–69	Total
Aortic valve disease				
AS	2	1	7	10
ASAR	2	12	5	19
AR	3	12	9	24
Combined	3	3	1	7
Mitral valve disease				
MS	—	10	8	18
MSMR	—	11	13	24
MR	1	6	9	16
Total	11	55	52	118

AS = aortic stenosis AR = aortic regurgitation MS = mitral stenosis MR = mitral regurgitation

combined with other heart diseases Angina pectoris occurred in one case in age group 16–29 years in 13 cases in group 30–49 years and in six cases in the older age group

Angina pectoris was much less common in the group with mitral valve disease in which it occurred only in 16%

In the majority of cases the coronary arteriograms were normal whether there was typical atypical or no angina pectoris Only in two cases was a significant coronary artery disease found

Table II *Arteriographic classification of coronary artery disease*

Grade 0	No visible changes or very small irregularities in the wall
Grade 1	Narrowing of the lumen in at least one place but less than 50 of the diameter of the artery
Grade 2	Narrowing of the lumen in at least one place by more than 50 of the diameter but no delay in filling of the distal branches
Grade 3	As 2 but with delayed filling of branches distal to the stenosis
Grade 4	Total occlusion

The three major arteries right coronary artery left anterior descending branch and left circumflex branch are considered separately and grouped according to the classification above

Case M H

This was a 54 year old woman with aortic stenosis, and a peak systolic gradient of 115 mm Hg She also had systemic arterial hypertension but no angina pectoris. Coronary artery disease was found at arteriography 2-2-? She died during an operation with insertion of a valve prosthesis Necropsy showed narrow orifices of the coronary arteries and many arteriosclerotic plaques in their distal parts

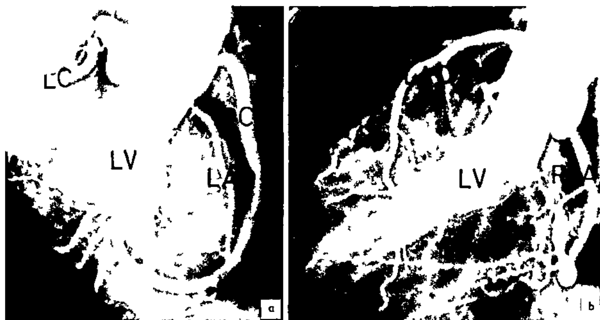


Fig 1 A 41 year-old woman with combined aortic stenosis (pressure gradient 800 mm Hg at rest) and insufficiency and angina pectoris Coronary arteries normal RCA = right coronary artery LAD = left anterior descending branch left coronary artery LC = left circumflex branch LV = contrast in left ventricle regurging from aorta *a* Left anterior oblique projection *b* right anterior oblique projection

Table III *Distribution with respect to angina pectoris and coronary artery disease*

Diagnosis	Angina pectoris	Coronary artery disease					
		0-0-0	0-0-1	0-1-1	1-1-1	2-[illegible] 2	1-1-3
Aortic valve disease	Typical	15	2	—	3	—	—
	Atypical	10	—	—	1	—	1
	Absent	25	—	1	1	1	—
Mitral valve disease	Typical	6	1	2	—	—	—
	Atypical	10	1	—	2	—	—
	Absent	29	4	4	—	—	—

For explanation of Figs 1–3 see Table II The three figures refer to the three main arteries right left anterior descending and left circumflex In this table however the order of the figures is at random

Case F K

A 5? year-old man with pure (grade 4) aortic regurgitation, an enlarged heart (730 ml/sq m) and increased left ventricular filling pressure (33 mm Hg) Coronary arteriography disclosed slight to severe changes (1-1-3) On a pre-operative exercise test he had angina pectoris but no typical ECG reaction A ball valve (Starr Edwards) was inserted with a good result. Three years later he had a myocardial infarction

These cases with coronary artery disease may be compared with a case with normal arteriograms

Case E E

A 41-year-old woman with combined aortic stenosis and regurgitation, with a pressure gradient at rest of 80 mm Hg. She had angina pectoris but the coronary arteries were normal (Fig 1) She was operated on with the insertion of a Cutter prosthesis Two years later she was markedl improved but had atypical chest pains.

In all 60 cases with aortic valve disease the surgical records were studied and findings concerning the coronary arteries were noted Only in three cases was some discrepancy found In these cases the arteriograms showed no changes (0-0-0) *in two* and slight changes (1-1-1) in one case All three had narrowing of the right coronary artery at its origin from the aorta which in two cases was caused by a calcified spur before the ostium This prevented the insertion of a cannula for coronary perfusion during surgery

In 23 cases out of the total material of 118 cases necropsy was performed In only two cases were slight to moderate narrowings found at autopsy when arteriography was normal

DISCUSSION

The frequency of *angina pectoris in so-called* rheumatic heart disease cannot be stated from our figures since the material is selected Our cases have mostly been referred to us from other hospitals in all parts of Sweden Many cases with typical angina pectoris may not have been sent to Uppsala for evaluation of their valvular heart disease since it was felt that a coronary heart disease was the main problem Even if we have declined neither operation nor hemodynamic and angiocardiographic evaluation in such cases it must be admitted on the other hand that coronary angiography has been performed more often in cases with chest pains

Nevertheless our figures compare well with those published by others concerning the frequency of angina pectoris in aortic stenosis (1 7 9 13 21) aortic regurgitation (6 17) and mitral valve disease (10 19 20)

The etiology of chest pain in valvular heart disease has been much discussed In necropsy studies the usual report has been normal coronary arteries (9 13 19) though the opposite has also been claimed (12) In a series of patients with valvular aortic stenosis it was found that the coronary arteries were as a rule normal or showed only slight changes (7) Obviously large differences are to be expected between clinical surgical and pathological series of patients Further even in necropsy series the age of patients with rheumatic heart disease might be expected to increase which might raise *the frequency of coronary ar*tery changes

Only few arteriographic studies in man seem to have been published Hale et al (11) claim that angina pectoris in valvular heart disease is usually due to coronary artery disease However they had *investigated five* cases with aortic or mitral stenosis and angina pectoris Only one case

had severe aortic stenosis and his coronary angiograms were normal Beverungen and Dux (2) studied 170 cases and found in 18 % changes which they considered pathological The highest frequency was noted in mitral stenosis 29 4 % Angina pectoris was present in 40 % of those with coronary artery changes As the degree of occlusion is not clearly stated and not graded it seems to be difficult to draw any more general conclusions from their results Storstein and Efskind (18) found normal coronary angiograms in 30 out of 38 cases with aortic stenosis

In studies of th coronary circulation in valvular heart disease no conclusive results have been reported (3 5 14 15 16) Recently Fallen et al (8) have studied the mechanism of angina pectoris in aortic stenosis with analyses of the coronary blood flow and the myocardial lactate production at rest and during isoprenaline infusion Though their groups of cases with angina pectoris and aortic stenosis were small they found no differences at rest b tween those with or without coronary artery disease During isoprenaline infusion both groups increased the myocardial lactate production but the coronary artery flow increased only in the group with coronary artery disease Though th ir seri s are small the results seem to confirm that angina pectoris in aortic stenosis has another mechanism than in coronary heart disease

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BETA RECEPTOR BLOCKADE IN MAINTENANCE OF SINUS RHYTHM OBTAINED BY ELECTROCONVERSION

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Abstract The effectiveness of beta receptor blockade as maintenance treatment of sinus rhythm obtained by electroconversion has been evaluated Out of 98 consecutive patients with chronic atrial fibrillation 74 were converted and controlled at regular intervals After three months only 27 of the 74 patients remained in sinus rhythm This gives a maintenance rate of 36 %

It is concluded that maintenance treatment with beta receptor blockade is ineffective This conclusion is discussed in the light of pharmacological effects of quinidine and beta receptor antagonists and of recent evidence on vagal stimuli as important for occurrence as well as persistence of atrial fibrillation

The new beta receptor blocking agent H 56/28 (Aptin® from AB Hassle Goteborg Sweden) was employed in the study Heart failure was provoked in a case of myocarditis Bradycardia made it necessary to discontinue treatment with the agent in three additional patients Aside from this no unwanted effects of H 56/28 were observed

Electroconversion of chronic atrial fibrillation (13) carries a high rate of immediate success which contrasts sharply with the poor long term results (3 4 12 15 16)

The value of preventing relapse by means of treatment with antiarrhythmic agents mainly digitalis and quinidine has not been settled (4 6 7 15 21)

As beta adrenergic receptor blockade offered a new approach to the problems regarding maintenance therapy and the effectiveness of such treatment had not been conclusively evaluated (18 19 20) the present study was undertaken

The new beta receptor blocking agent H 56/28 (Aptin®) was selected for the trial because of its interesting properties of providing additional stimulation of the beta adrenergic receptors of the heart (22)

MATERIAL AND METHODS

A total of 10 patients consecutively referred for electroconversion were originally enclosed in the study Later on four patients were omitted because of various complications leaving 93 consecutive patients with atrial fibrillation for the final study (Table I) As will be observed a majority of the patients were females and the heart disease was of rheumatic origin in two third of the series The remainder suffered from coronary hypertensive and congenital heart disease

The indications for electroconversion were kept rather wide in accordance with those employed at the department during similar studies with quinidine (3)

Digitalis medication was routinely stopped 3– days prior to electroconversion and treatment with H 56/28 simultaneously started The latter agent was given by the oral route in a dosage of 40 or 50 mg four times daily Following electroconversion digitalis medication was resumed in all the patients In those who obtained sinus rhythm H 56/28 was continued in the same dosage for three months or until relapse

RESULTS

Of the 98 patients with chronic atrial fibrillation subjected to electroconversion 74 obtained sinus rhythm giving a restoration rate of 76 which was equal for both sexes For cases with atrial fibrillation of less than five years duration the conversion rate amounted to an average figure of 90 % while the corresponding figure decreased to 53 for cases with duration of more than five years In 90 patients with a heart volume of less than 800 ml per square metre sinus rhythm was established in 69 or in 77 In eight patients with heart volumes above this size conversion was successful in only two

The main result of the present study (Table II) is unequivocal Maintenance of sinus rhythm by means of beta receptor blockade is an ineffective

Table I *The series of patients with chronic atrial fibrillation subjected to treatment with H 56/28*

Sex distribution	No of pats	Mean age (y)	Rheumatic heart dis	Other heart dis
Female	63	52	45	18
Male	35	52	18	17
Total	98	52	63	35

Table II *The effectiveness of H 56/28 in maintaining sinus rhythm after electroconversion of chronic atrial fibrillation*

Sex distribution	Sinus rhythm after DC shock	Sinus rhythm after 3 mo (no)	( )
Female	47	16	34
Male	27	11	40
Total	74	27	36

Table III *Comparison between treatment with H56/28 and quinidine in maintaining sinus rhythm following electroconversion*

	Sinus rhythm after DC shock	Sinus rhythm after 3 mo (no)	( )
H 56/28	74	27	36
Quinidine (3)	185	96	52

Table IV *Comparison between two series of patients who failed to maintain sinus rhythm on treatment with quinidine or H 56/28 after electroconversion In connection with the second electroconversion the treatment was changed from quinidine to H 56/28 and vice versa*

Treatment changed from	No of pats	Sinus rhythm after DC shock	Persistence of sinus rhythm (3 mo)	(6 mo)
Quinidine to H 56/28	22	16	3	0
H 56/28 to quinidine	22	19	9	6

treatment This becomes obvious by comparison with a similar study carried out at the department with quinidine as maintenance treatment (Table III)

Included in the series were two groups of patients who failed to maintain sinus rhythm after conversion on treatment with either quinidine or H 56/28 Accidentally the two groups became evenly sized At the second conversion the treatment was changed from quinidine to H 56/28 and vice versa Again quinidine proved to be the best treatment although the series is too small for statistical analysis (Table IV)

No major complications were encountered during treatment with H 56/28 (Aptin) A middle aged man initially considered to suffer from lone fibrillation developed frank right heart failure Subsequent examination including heart catheterization revealed that he suffered from myocarditis For this reason H 56/28 was withdrawn and treatment with digitalis and diuretics instituted In three other patients successive increase of the PR interval and concomitant bradycardia made it neccessary to discontinue the treatment Serial estimations were made of blood counts and function tests of liver and kidneys No changes occurred which could be ascribed to the use of H 56/28

COMMENTS

Beta adrenergic receptor blockade has been widely employed as antiarrhythmic principle but has received surprisingly little attention as maintenance therapy of sinus rhythm obtained by electroconversion Only a few reports are available from the current literature Thus Tsolakas et al (19) employed propranolol in 18 patients after conversion and noted a relapse rate of 50% in less than two months They consequently regarded the treatment as ineffective Similarly Szekely et al (18) tried quinidine procainamide and propranolol given singly or in combination and found identical recurrence rates Unfortunately their report does not provide the figures upon which their conclusions are based According to unpublished observations (20) beta receptor blockade given singly or in combination with atropine has not yielded encouraging results

No valid conclusion could be drawn from the above information and it seemed justified to undertake the present trial

This study distinctly shows that beta adrenergic receptor blockade is ineffective in maintaining sinus rhythm after electroconversion and com

parably less effective than quinidine as employed by Bjerkelund et al (3) As these two trials were carried out at the same department and under identical circumstances this conclusion is warranted

However the result has to be considered in greater detail for two reasons Firstly quinidine is not an innocuous drug but may produce dangerous side-effects Secondly there exists conflicting evidence as to the effectiveness of quinidine in maintaining sinus rhythm Thus Korsgren et al (12) employed quinidine and found sinus rhythm to persist in 43% of the patients during an observation period of from 4 to 19 months In the study by Halmos (8) the respective figure was 55% after three months and no significant difference was found between the untreated group and two other groups treated either with quinidine or with effervescent potassium A similar conclusion was reached by Coelho et al (4) in a controlled study After three months quinidine was slightly better than no treatment whereas no significant difference could be observed after six months Other authors have published series (6 7 16 21) which raise serious doubt as to whether or not quinidine treatment is better than no treatment Comparison with series based upon conversion as well as maintenance treatment with quinidine cannot be made without reservations because those converted have already demonstrated a special response to quinidine However it is to be noted that Rokseth (17) in his series found persistence of sinus rhythm in 61% after six months and Cram r (5) in 51 and 48% after three and six months respectively

After all it is obvious that beta receptor blockade is less effective in maintaining sinus rhythm than quinidine This is a surprise considering the marked resemblance between the beta receptor blocking agents and quinidine They have in common a local anaesthetic property and furthermore they increase the fibrillation threshold and reduce the conduction velocity of the heart chambers (9 10) However one important difference exists Quinidine possesses a significant anticholinergic effect which facilitates sino-atrial and atrio-ventricular conduction whereas beta receptor blockade has the opposite effect That this difference of action plays an important role is supported by both clinical and experimental data

Firstly quinidine is effective in converting atrial fibrillation to sinus rhythm while beta receptor blockade is not (9) Secondly quinidine facilitates and beta receptor blockade inhibits sino atrial as well as atrio-ventricular conduction These are well known clinical effects Thirdly experimental data have strongly pointed to vagal function as being of prime importance in the pathogenesis of atrial fibrillation (2 11 14) This has recently received substantial clinical support by the elegant studies of Balsano et al (1) If correct the concept of vagal function as important for production as well as for maintaining atrial fibrillation may explain the results of the present trial Quinidine would counteract the vagal stimuli beta receptor blockade would indirectly augment them by ablation of the sympathetic tone

The present study also shows the value of beta receptor blockade as pretreatment for electroconversion The conversion rate was 76% as compared to 83% in the series of Bjerkelund et al (3) who employed quinidine for the same purpose The corresponding figure was 78% in the series by Halmos (8) who did not give any pretreatment Although the initial results of electroconversion are uniformly very good it thus seems that beta receptor blockade may have an adverse effect also in this respect

The beta receptor blocking agent H 56/28 (Aptin) employed in this study produced no serious side-effects This was in contrast to experience with propranolol under the same circumstances (10) The precipitation of heart failure in a case of myocarditis is not surprising since this condition is a well known contraindication for such treatment It is more surprising that heart failure did not occur more frequently in this series Probably this was due to the property of H 56/28 to exert also a stimulation of the beta receptors of the heart (22)

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THE ELECTROCARDIOGRAPHIC DIAGNOSIS OF LEFT VENTRICULAR HYPERTROPHY

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Abstract In a patho anatomical study the accuracy of the usual ECG criteria of LVH has been tested in 95 patients above 50 years Bundle branch block myocardial infarction and isolated right ventricular hypertrophy were excluded. According to the heart weight and the thickness of the free left ventricular wall the material was divided into a group with pronounced left ventricular hypertrophy (LVH) and a control group showing no e id nce of LVH The anatomically uncertain cases were gathered in an additional group

S + R $_{-6}$ > 35 mm and S_m + $_{Rm}$ V $_{-6}$ > 40 mm were the most sensitive volta e criteria (70–85% in the LVH group) the frequency of positives in the control group be ng 11%

In diagnosing LVH the onset of the ventricular activation time (VAT) was superior to the QRS duration T enty five per cent of the patients with LVH had a ventricular activation time (VAT) ⩾ 0 06 while as many as 80 had VAT ⩾ 0 05 The numbers of pos tive values in the control group were 0 and 7 respectively

ST depression together with inverted T wave in the lateral precordial leads was seen in 65 of the LVH group and in 4% of the control group

Based on the results of the present study various combinat ons of criteria for the diagnosis of LVH ha e been discussed

Left ventricular hypertrophy (LVH) has long been known to give rise to certain characteristic changes in the electrocardiogram The findings are most pronounced in the left precordial leads and the mo t accepted criteria are 1) increased voltage of the QRS deflection 2) delayed ventricular activation time and 3) S–T segment depression and T wave inversion The first two criteria ar related to increased ventricular mass ST–T changes are less specific indicating myocardial disease (10) However they are also said to be a result of altered ventricular depolarization due to hypertrophy (9) The accuracy of these criteria has been based on clinical findings alone (6 14 16) or on patho anatomical correlations (1 7 10 13) The anatomical findings of LVH have b en based on the thickness of the free left ventricular wall and/or the total or left ventricular heart weight The upper normal limit of the left ventricular wall has been stated to be 10–15 mm (2 12) and the normal heart weight 250–400 g Nielsen (11) found the mean thickness of the free left ventricular wall of older people to be 13 8 mm Their mean heart weight was 330 g Considerable discrepancies exist however as to the thickness of the free left ventricular wall consistent with LVH It is often uncertain how the measurements have been performed Some authors define LVH as existing if the ventricular wall exceeds 12 mm (1 13) On the other hand 15 mm has also been used as the upper normal limit (7) We find it difficult to choose an exact anatomical limit there must be borderline cases

The aim of the present study has been to test some of the usual ECG criteria of LVH This has been done with three groups of patients one having pronounced LVH on anatomical examination the other showing no evidence of LVH and the third comprising borderline cases

MATERIAL AND METHODS

The 95 patients included in this study died in the medical departments VIII and IX of Ullevål Hospital Os o during 1966 They were all more than 50 years and had had a 12 lead ECG recorded at least once during the last 6 weeks prior to death The following cases were exclud d ( ) ECGs showing bundle branch block (*b*) myocard al i farct on, whether pro ed by the ECG or at autop y and (*c*) isolated right entricular hypertrophy (fre right ventricular wall > 5 mm and fr e lef

Table I *Patho anatomical criteria for grouping patients*

Group	Thickness of *free left* ventricular wall (mm)	Heart weight *exceeding mean* According to Zeek (17) ( )
Left ventricular hypertrophy	≥17	Without regard to the heart weight
	15 and 16	≥100
Border line		
I	*16*	*<100*
	15	50–100
II	14	Without regard to the heart weight
	13	≥50
Control	≤13	<50

ventricular wall <14 mm) We have not deliberately excluded cases with ECGs possibly obscured by digitalis therapy or electrolyte disturbances nor have we excluded cases having myocardial fibrosis or severe coronary atheromatosis Cases with combined hypertrophy have been included

The patho anatomical examinations were performed in *the Department of Pathology Ullevål Hospital* The heart weights were recorded according to the autopsies as registered by the different prosectors According to the body length of the patient the mean heart weight of Zeek s (17) was calculated The heart weight of the patient was then estimated as the percentual increase or decrease of the mean heart weight The left ventricles were opened by a longitudinal incision along the antero septal portion c rried along the apex and along the posterior septal portion as closely as possible at right angles to the epicardial surface The measurements of the ventricular walls were in all cas s performed by one of the authors The measurements were made to the nearest millimetre 2 cm below the annulus fibrosus. Care was taken not to include epicardial fat or part of the papillary muscles According to the heart weight and the thickness of the free left ventricular wall the material was divided into groups (Table I)

The ECG tracings were made on an Elema Schonander Mingograph 4? B with a paper speed of 50 mm/sec The ECGs were interpreted by the authors Every ECG should be accompanied by a *standard* 1 mV *equaling* 10 mm The upstrokes and downstrokes were measured to the nearest millimetre according to Wilson et al (15) The duration of the QRS in leads I–III and the ventricular activation time in V_{5-6} was measured to the nearest 0 01 sec ST and T wave changes are difficult to measure It is not difficult however to register the ST segment as normal i e not depressed or depressed nor *is it difficult to register the* T wave as *isoelectric* or negative We felt this way of recording the ST–T changes to be equally valuable as measurements expressed in mm ST depression with a concomitant negative T wave was recorded as a positive ECG finding The ECG criteria used to detect LVH are presented in Table II

RESULTS AND DISCUSSION

On anatomical examination there were 20 cases with LVH and 27 cases without LVH The mean age was 77 4 and 70 9 years respectively The borderline group comprised 48 cases 15 in group I and 33 in group II

Most authors agree that the ECG findings seen

Table II *ECG criteria of left ventricular hypertrophy and percentual number of positive cases*

ECG criteria	Per cent positive values			
			Borderline	
	LVH $n=20$	Control $n\approx 27$	Group I $n=15$	Group II $n=33$
Left axis dev 0 –90 (10)	80	37	40	42
$R_I + S_{III} > 25$ mm (7 15)	25	0	7	0
$R_{VL} > 11$ mm $R_{VF} > 20$ mm (5)	30	4	7	0
$R_{V5-6} > 26$ mm (15 17)	40	4	0	3
$R_{V6} > R_{V5}$ (3)	45	15	33	0
$S_{V1} + R_{V5\ 6} > 35$ mm (15)	70	11	20	6
>40 mm (11)	85	11	27	6
S_m R_m $V_{1-6} > 45$ mm (11)	70	11	14	6
>50 mm (11)	55	4	0	0
Ventricular				
activation ≥0 06 mm (17)	25	0	7	0
time ≥0 05 mm (15)	80	7	33	9
QRS duration ≥0 10 mm (17)	45	4	7	12
≥0 11 mm (17)	20	0	7	0
S–T depression *and*				
negative T in $V_{5\ 6}$ (6)	65	4	14	3

in LVH are caused by increased ventricular mass and not by an underlying myocardial disease (9) As digitalis therapy and chronic myocardial disease may affect the ECG especially the ST–T segment an evaluation of the frequency of digitalis therapy and death due to chronic myocardial disease was essential In the LVH group 60% of the patients were digitalized and 30% died in heart failure whilst in the control group 22 were digitalized and none died in heart failure Among the patients receiving digitalis we could not demonstrate a higher frequency of ST-T changes consistent with LVH

Table II presents the ECG criteria of LVH frequently used and the percentual number of positive ECG findings in the different groups

The values of the borderline group II and the normal group are very much the same The anatomical criteria may therefore be changed from those of the normal group to those of borderline group II (Table I) without increasing the frequency of positive ECG findings It may be concluded that from an electrocardiographic point of view borderline group II may be regarded as normal

Th remaining part of the borderline group group I comprises those cases probably having LVH anatomically The percentual number of positive ECG findings in this group is higher than in the normal group and lower than in the LVH group This group really may be looked upon as a borderline group Other investigators have used the same criteria of LVH as we have for borderline cases group I This may explain th higher number of positive values in the LVH group in the present study

LVH usually shows left axis deviation (14) In the present study this occurred in 80% of the LVH group However 37% of the control group had left axis deviation

Even though the ECG criteria of LVH ought to be met with frequently in the LVH group many of the voltage criteria ($R_I + S_{III} > 25$ mm $R_{aVL} > 11$ mm or $R_{aVF} > 20$ mm $R_{V5\ 6} > 26$ mm and $R_{V6} > R_{V5}$) were present in less than 50 The number of false positive values were however also low $S_{V1} - R_{V\ 6} > 35$ mm and maximal R + maximal S in $V_{1\ 6} > 45$ mm were more sensitive criteria The most sensitive voltage criterion is $S_m + R_m$ V_1 > 40 mm This occurred in 85% of the LVH group As regards this criterion the frequency of false positives was 11% in the control group and 6% in the borderline group II

The exact duration of the ventricular activation time (VAT) i e from the beginning of the Q wave to the peak of the R wave is difficult to measure In the present study only 25% of the patients with anatomical LVH had a VAT $\geq 0\ 06$ while as many as 80 % had a VAT $\geq 0\ 05$ The number of false positives were 0 and 7% respectively Accordingly with VAT $\geq 0\ 05$ there is a great increase in sensitivity with only a correspondingly small decrease in specificity It therefore seems reasonable to regard a VAT $\geq 0\ 05$ as a positive criterion in the diagnosis of LVH

In the present study the VAT seems superior to the QRS duration as a sign of LVH Less than half of the cases of the LVH group had a QRS $\geq 0\ 10$ and not more than 55% had a QRS $\geq 0\ 09$

ST depression together with an inverted T wave in the lateral precordial leads is often called strain pattern These changes have not been measured only registered Consequently it is more difficult not to be biased We found these changes in 65% of the LVH group and in 4 of the control group

The present study supports earlier investigators there is no single good criterion for the diagnosis of LVH The criteria which are reasonably specific are not very sensitive and vice versa The number of correct diagnoses may be increased when different criteria are combined To diagnose LVH electrocardiographically one may demand one positive out of several specific criteria or all positive out of some unspecific criteria which we have called sensitive criteria Different combinations of criteria have been tested on the basis of the results of the present study The three criteria which seemed to be most valuable are listed below They may be used as a sensitive (1 point) or as a specific criterion (2 points)

$S_m - R_m$ in $V_{\ 4} > 40$ mm (1 point)
$S_m + R_m$ in $V_{\ 6} >$ 0 mm (points)
Ventricular activation time in $V_{5-6} = 0\ 05$ (1 point)
Ventricular activation time in $V_{5-6} \geq 0\ 06$ (points)
ST depression together with T inversion in V_{5-6} (? points)

When the score was applied to the 20 cases in the LVH group and to the 60 cases in the control group and borderline group II the following was found

0 point no cas s in the LVH group in controls 85%
1–2 points 25% in the LVH group in controls 15
3–6 points 75 in the LVH group in controls none

The practical use of the results has som limi tations only people above 50 years have been examined The normal QRS voltage is higher in younger people and unspecific ST–T changes are probably less common

Changes due to myocardial infarction will of en interfere with the ECG criteria of LVH In this study these cases have been excluded Previous myocardial infarction may be unknown however and precautions may not be taken

Bundle branch block which is often seen in LVH has been excluded

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INTRAVASCULAR COAGULATION AND HAEMORRHAGIC PANCREATIC NECROSIS IN A PATIENT WITH CARCINOID DISEASE

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Abstract A case with haemorrhagic pancreatic necrosi due to occlu ion of the common hepatic artery is de scribed It was fol owed by a decrease in platelets shor tened coagulation time in plastic tubes low thrombotest decreased prothrombin + proconvertin activity factors V IX and fibrino en All these changes are similar to those occurrin after injections of thrombin i e during intra vascular coagulation

Intravascular coagulation may occur in a number of dis ases and play a role in the appearance of various symptoms and signs in clinical conditions (7 8 10) Thus the importance of the vascular syst m in the pathogenesis of pancreatic necrosis was suggested as early as 1862 by Panum who produced the lesion by injecting wax particles into the arteries of the pancreas (9) Ligation of some of the arteries alone failed to induce pan creatitis whereas this could be achieved by the combination of trauma and ligation (12)

Many of the various forms of trauma used to produce experimental haemorrhagic pancreatitis (cf 12) are also able to trigger platelet damage or intravascular coagulation

An account is given in the following of a case in which intravascular coagulation and haemor rhagic pancreatic necrosis occurred in association with carcinoid disease

CASE REPORT

The patient was a 53 year-old man with a history of a bleeding ulcer in 1941 In 1958 attacks of flushing started and appeared 2–3 times a year The diagnos s of ca cinoid disease was established in 1961 when the 5 hydroxy indole acetic acid excretion was 4?4 534 mg/ ?4 h as compared to the normal value of 4 8 ± 2 4 mg in 30 medical students of both sexes

The primary tumour of the ileum wa remo ed in 1964 and the diagnos s confirmed h stologically

The patient s symptoms became successi ely wors and more frequent in 1965–1966 He had strong flushing and cyanosis especially of the face tachycardia headache profuse sweating dyspnoea on exertion as well a attacks durin the ni ht diarrhoea and 15 micturitions per 4 h Severe itchin occurred after taking a hot bath (Sansert® (Ciba) was tried without effect) Selective angiography of the hepatic and coeliac arteries showed numerous small vascular formations in the liver resembling metastases He was admitted for possible local Purinethol® treatm nt of the liver metastases.

Operation was perform d on Aug 26 1966 but durin dissection the common hepatic artery ruptured and could not b repaired Pos operative y his blood pressure wa initially labile Metabolic acidosi de eloped and despite numerous blood transfusions and attempts to correct the electrolyte balance the patient died two days later pre sentin a picture of circulatory failure

Autopsy

The iliac trunk was opened from the aorta The left gastric artery and the splenic artery were intact. The hepatic and gastroduodenal arteri s branched just on the borderline of the operative region. The former artery was l gated at the site of bifurcation The gastroduodenal artery was partially strictured and thrombos d but with the lumen st ll pa ent A fresh venous thrombus was present in the splenic vein. The duodenum includin the head of the pancreas was haemorrhagic and necrotic The liver weighed 4700 g and was infiltrated by carcinoid metastases.

Coagulat on stud es

The results of coagulation analyses before and after operation are given in Table I There was a fall after operation in platel t count, shortened coagulation time in plastic tubes decreased thrombotest and prothrombin procon ertin activities low factors V and IX and fibrino en le e s No fibrinolysis was observed (Methods cf (6))

DISCUSSION

Since the coagulation factors during operation do not change the interest in this case centres around

Table I *Blood and coagulation data before and after operation Aug 26 1966*

Da e	18 8	24 8	27 8	28 8	Normal value
r ding time	1 20	1 45			
oagulation time	5 15	4 30	2	5 30	4–8
gulation time in plastic tubes		19 15	11 15	14 15	20–30
atelets µl	232 000	226 000	70 000	100 000	200 000–400 000
nrombotest	100		16	30	
rothrombin + proconvertin		82	42	58	85–110
actor V		80	40		80–120
a tor IX			54		60–140
Fibrinogen per 100 ml		0 34	0 13	0 36	0 26±0 06
Fibrinolysis / per 100 ml		0	0	0	31±25
Haematocrite		46	39	46	
Haemoglobin g per 100 ml	14 1	15 6	13 3	14 6	
White blood cells µl	7200		17 400	9600	

his pancreatic disease It is well known that damage to the pancreatic vessels may produce pancreas necrosis and that haemorrhagic pancrea titis is accompanied by venous thrombosis in adjacent vess s presumably caused by the release of active ry n

In this c he decrease in fibrinogen and other coagul n factors could be due to im paired p odu tion r increased consumption The first exp n tion unlik ly because fibrinogen increase o 0 13 o 0 36 g per 100 ml on the second d o tion The latter is indicated by the l s and blood coagulation factors

The pres agulation picture is similar to that o n vascular coagulation in duced by t ir endotoxin antigen antibody ctions a ir injuries in experimental imals (2) or obstetric shock (11) inant purp (4) giant haemangioma (1) and generaliz d h em rrhagic purpura due to vaccinia (7) and biliary cirrho is with bleeding tendency in man (6)

The release of trypsin and the thereby induced platelet aggregation and intravascular coagulation in pancreatic vessels may well be one cause of the total necrosis of the gland often found after small traumas

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A REVIEW OF 191 PATIENTS WITH MYOCARDIAL INFARCTION TREATED IN A SWEDISH CORONARY CARE UNIT

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Abstract The first Swedish Coronary Care Unit was opened in Borås in 1966 with six beds During the first 17 months 191 patients with confirmed myocardial infarction have been treated Of these 72 (37 7) have died This is a high mortality rate compared with that of several other recently publish d series but we consider that the difference is due *partly* to the fact that the figures refer to the first months that the department was in operation and *partly* because other series consisted of selected patients The immediate cause of death has been determined in each case At this stage our impression is that, with adequate observation and early treatment of arrhythmias, unexpected circulatory arrest seldom occurs At present, in almost all cases the cause of death is progressive cardiac failure

Since 1963 when Brown published the first series from a Coronary Care Unit (CCU) the number of such units throughout the world has rapidly increased and many series of cases have been published primarily from North America and Great Britain (2 3 5 7 8 10 12 13 14 15 17 18)

Our series includes 191 patients with myocardial infarction In addition to these cases patients with suspected infarction or serious cardiac irregularities have also been cared for in the CCU Our series is of interest partly because it is the first from a Swedish CCU (4 16 20) and partly because of the method of selection We shall discuss the difficulties in comparing the mortality rates in different series and shall attempt to justify our opinion that the most important function of such a unit is to prevent the occurrence of dangerous cardiac arrhythmias

Coronary Care Unit (CCU)

In 19 6 the medical department of the Central Hospital Borås, opened an observation ward for pati nts admitt d with suspected infarction The ward contain s x beds and the patients are separated by metal screens A central observation station is placed so that the nurse and the patient can see each other An oscilloscope provided with a heart rate meter and a threshold alarm is continuously connected to each patient At the nurse s station there is a graphic ECG apparatus which can instantly record the ECG from any patient desired Immediately adjacent to the nurse s station is a resuscitation centre equipped with two DC defibrillators a Bennet Ventilator plus the necessary equipment for the treatment of circulatory arrest A portable X ray machine is also stationed in the ward The chief of the department is a junior consultant and an experienced registrar is responsible for the day to day running of the department At night, the physican-on-duty whose room is immediately adjacent to the CCU is responsible for the ward When necessary an anaesthetist can be quickly summoned During the 24 hours the pati nts are continuously under observation by specially trained nurses

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Monitoring and treatment principles

All patients suspected of having a myocardial infarction are treated primarily in the casualty department where any necessary immediate treatment s started after which the patient is transferred as soon as possible to the CCU If circulatory arrest occurs in the casualty department or has occurred before admission treatment is started immediately on the ame principles as those applied in the CCU If regular card ac action i restored the patient is transferred as quickly as po sible to the CCU for continued observation and treatment There the patient is placed in a bed which allows rapid alterations in position An oscilloscope s connected at once ECGs are not only taken repeatedly th oughout the 24 hours but also immediately on any change in the pat ent cond tion or if an ar hythmia is suspected Rout ne pulse and blood pressure are recorded at hourly inte als Mob li a tion s not car ed out in the acute phase Salt restriction s not enforced a normal diet is followed Routine oxygen is g en v a a nas l catheter If pain is present, peth dine is adm n ste ed n doses of 25 50 mg, sometimes intra v nous y D oumarol is given as a rule In cases of manifest ca diac failure the pat ent is d gitalised intravenously and d u et cs are gi en when nece Supraventricular arrhythmias with a rapid y

Table I *Sex distribution and distribution between survivors and nonsurvivors*

	Alive	Dead	Mortality ( )
Men	85	44	32 5
Women	34	28	45 0
Total	119	72	37 7

rate are treated with intravenous digitalis but should this fail to prevent the development of a haemodynamically unfavourable situation we administer a counter shock at an early stage with the patient anaesthetised In cases of ventricular arrhythmias we usually first give procaine amide either intravenously or orally and secondly chinidine During the first months that the CCU was in operation there was no regular routine for the treatment of ventricular extrasystoles Now our routine is to use lidocaine a th rug of choice AV block of grades II and III is r ated initially with isoproteronol infusion but if thi th ry is inadequate an electrode is inserted for conne u c a pacemaker Countershock is administered in s of ventri ular tachycardia whereas ventri ular fi ution is treated as quickly as possible by defibrillat c ried out by either a nurse or a doctor If no immedi ult is obtained the treatment is the sam as fo a i external cardiac compression artificial re n and the administration of sodium bicarbonate la resort in cases of asystole our routine i t o timulate the heart electrically via electrodes r an thoracically The treatment of shock has r

Present seri s

From September 966 ntil the end of January 1968 191 patients w h n fi med myocardial infarction have een treated n t In order to have the best sible oppo t i e s the problems associated h ardi infa r e h e considered it necessary ha e all patients w th the slightest suspicion of acute coronary o lusion under lo e observation We have therefore not set n i e limits All patients admitted within three days of the nset f symptoms were included Patient admitted with an irreversible circulatory arrest were excluded Th diagnosis of myocardial infarction has been made on the ba i of the WHO criteria 1959

1 Pathological Q wave followed by an elevated ST segment and an inverted T wave

2 ST elevation and T wave changes indicative of an infarction followed by a significant but transient in rease in GOT (more than 50 U) or

3 Left bundle branch block foll wed by similar enzyme changes as in 2

All the patients who died except two (97%) were subjected to a postmortem examination All patients who were admitted with circulatory arrest and regained a stable cardiac rhythm following treatment were immediately transferred to the CCU (10 patients) Of the

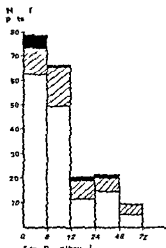


Fig 1 Interval between the onset of symptoms and arrival at hospital ☐ 1st infarction //// nd infarction ■ 3rd infarction

191 patients with myocardial infarction 129 (68) were men and 62 (3) were women In six cases it was not po sible to e tablish a diagnosis of infarction on clinical grounds but at the postmortem fresh infarctions were revealed In two cases with an established diagnosis of infarction the subsequent postmortem failed to reveal a fresh infarction In one of these cases death occurred within 6 hours of the onset of symptoms and no microscopic examination of the myo ardium was made The two patients on whom a postmortem was not performed had clinically established infarctions

Table I shows the sex distribution and that for survivors and nonsurvivors

The duration of stay in the cardiac infarction unit was decided according to the patient s condition In the absence of complications the patients were as a rule under observation for 4-5 days

RESULTS

The interval between the onset of symptoms and the patient s admission is apparent from Fig 1 47% were admitted within 6 hours 75% within 12 hours and 86% within 24 hours Of those who died 43 % were admitted more than 12 hours after the onset of symptoms whereas only 23% of those who survived were admitted after more than 12 hours

Table II *Average age of survivors and non survivors*

	1st time myocardial infarction	2nd time myocardial infarction	3rd time myocardial infarction	Total
Alive	64 (41-86)	66 (39-83)	—	64
Dead	68 (51-85)	70 (53-83)	68 (58-87)	69

Table III *Mortality in different age groups and in relation to the number of infarctions*

Age group	1st time myocardial infarction		2nd time myocardial infarction		3rd time myocardial infarction		Mortality rate
	Total	Deaths	Total	Deaths	Total	Deaths	
30–39	—	—	1	—	—	—	—
40–49	8	—	—	—	—	—	—
50–59	37	10	6	2	2	2	14 (31)
60–69	41	9	16	11	2	2	22 (37)
70–79	49	18	14	10	2	2	30 (46)
80–89	7	2	5	3	1	1	6 (46)
Total	142	39 (27)	42	26 (62)	7	7 (100)	72 (37 7)

Frequency of complications on admission

Table IV shows the complications present on admission Survivors and nonsurvivors have been shown separately and moreover the number of infarctions the patients have had is given Complications such as cardiac failure shock or circulatory arrest have been uncommon on admission among the survivors (11) but common among nonsurvivors (50 %)

Of the seven patients who have had their third infarction four were admitted in a state of circulatory arrest and one was in severe shock In all ten patients were suffering from circulatory arr st (ventricular fibrillation) on admission and although in all of these it was possible to restore cardiac action nine of them have subsequently died and only one has been discharged

Mortality

Table III shows the mortality during the stay in hospital (as a rule 3 weeks) in different age groups and in relation to the number of infarctions the patients had suffered Seventy two patients (37 7%) died 44 men and 28 women

The mortality was lower for men (34%) than for women (45%) but the difference is not statistically significant Table II shows that the average age for nonsurvivors (69 years) is higher than for the survivors (64 years) There is no significant difference between the ages of patients with a first second or third infarction whereas there is a marked difference in mortality 27 5% for those with a first infarction 62% for those with a second and 100 % for those with a third Of the 72 who have died 33 (47) had had at least one previous infarction

Cause of death and survival time

Table V shows the length of survival in days for the 72 patients who died (54 % within 2 days and 75% within 5 days) Only nine patients died after they had left the CCU Of these three had be n transferred to another department because of residual brain damage following restoration of the circulation (persistent coma) two had been transferred because of progressive heart failure and only four patients in the whole series died after they were no longer considered to be in need of

Table IV *Complications on admission*

	Rate of complications on admission in 72 patients who died in myocardial infarction				Rate of complications on admission in 119 patients who left hospital after myocardial infarction			
	VF AS	Failure	Shock	Total	Asystole	Failure	Shock	Total
1st time myocardial infarction	4	8	4	16 (41)	1	11	1	13 (13)
2nd time myocardial infarction	3	9	2	14 (54%)	—	1	—	1
3rd time myocardial infarction	2	3	1	6 (86%)	—	—	—	0
Total	9	0	7	36 (50%)	1	12	1	14 (11%)

Table V *Time of death in CCU and in ward*

Figures within parentheses indicate death in ward

Day	1st time myocardial infarction	2nd time myocardial infarction	3rd time myocardial infarction	Total
1	13	10	2	25
2	6	2	1	9
3	4	1	2	7
4	3	2	0	5
5	3 (?)	0	0	3
6–7	4	5	0	9
8–9	3 (1)	2 (1)	0	5
10–14	1	3 (1)	1 (1)	5
21–28	2 (2)	1	1 (1)	4
Total	39	26	7	72

Table VI A *Cause of deterioration and death in 72 nonsurvivors*

Cause of deterioration	Total	Cause of death	Total
Left vent failure	23 (32)	Left vent failure	19 (26)
Shock	22 (30)	Shock	3 (4*)
Asystole	5 (7)	Asystole	26 (36)
Ventricular fibrillation	19 (?6)	Ventricular fibrillation	1 —
Cause unknown	3 (4)	Cause unknown	10 (14*)
		Rupture	9 (12*)
		Brain damage	4 (5%)

continuous observation The cause of death is not easily determined (Table VI A) In order to obtain an idea of the progress of the disease from the initial deterioration until death Tables VI B and C have been compiled which show the relation between the cause of deterioration (on clinical grounds) and that of death judged either clinically from the ECG or at the postmortem In the case of first and second infarctions the cause of the deterioration was commonly cardiac failure shock or ventricular fibrillation The 19 patients with ventricular fibrillation are of particular interest In nine of them ventricular fibrillation was present on admission and only ten of the patients who died developed ventricular fibrillation after admission Nine patients who developed ventricular fibrillation and were successfully defibrillated finally died in a state of asystole preceded in some cases by a recurrence of ventricular fibrillation Four of these defibrillated patients died from progressive cardiac failure and one has subsequently died of cardiac rupture Four of the defibrillated patients died of permanent brain damage after 1 2 9 and 14 days respectively These four patients were in coma though with spontaneous respiration following

Table VI B *Relation between cause of deterioration and death in first and second infarctions*

Figures within parentheses indicate death in ward

1st time myocardial infarction Cause of deterioration	No of pats	Cause of death	No of pats	2nd time myocardial infarction Cause of deterioration
Left vent failure	2	Left vent failure	3	Left vent failure
10 pats	4	Asystole	5	11 pats
	1	Shock	0	
	0	Rupture	1	
	3 (?)	Cause unknown	2	
Ventricular	2	Left vent failure	2	Ventricular
fibrillation	0	Ventricular fibr	1	fibrillation
7 pats	4	Asystole	4	9 pats
	1	Rupture	0	
	0	Brain damage	2 (1)	
Asystole 2 pats	0	Asystole	2	Asystole 2 pats
	1	Shock	0	
	1	Rupture	0	
Shock 18 pats	8	Left vent failure	0	Shock 2 pats
	2	Asystole	2	
	1	Shock	0	
	6 (1)	Rupture	0	
	1	Cause unknown	1	
Sudden death 3 pats	3 (3)	Cause unknown	0	—

Table VIC *Relation between cause of deterioration and death*

Figures with n parentheses indicate death in ward

3rd time myocardial infarction

Cause of deterioration	Cause of death: Left vent. failure	Asystole	Brain damage	Total
Left vent. failure	1	1	0	2 (1)
Ventricular fibrillation	0	1	2	3 (1)
Asystole	0	1	0	1
Shock	1	0	0	1
Total	2	3	2	7

defibrillation until death ensued In only one case was it impossible to defibrillate and the patient died in a state of intractable fibrillation

Patients who deteriorated in a state of shock died as a rule from cardiac failure or cardiac rupture It is probable that some of the nine cases of cardiac rupture were the result of external cardiac compression (iatrogenic rupture)

Complications among survivors

Table VII shows the complications noted among the 119 surviving patients with the exception of arrhythmias which are given in Table VIII It is apparent that cardiac failure is the most common complication Ventricular fibrillation was observed in only one case—i e the only patient discharged following ventricular fibrillation caused by myocardial infarction Two cases of cerebral embolism occurred in patients aged 65 and 67 presumably arising from a mural thrombus at the infarction site

Frequency of arrhythmias

The types and frequency of recorded arrhythmias are given in Table VIII Our method resulted in the recording of arrhythmia in 139 patients (73%)

Table VIII *Incidence of arrhythmias*

Type of arrhythmia	No of pats.
Supraventricular bradycardia	4
Supraventricular tachycardia	14
Supraventricular ectopics	14
Atrial flutter	1
Atrial fibrillation	34
Nodal rhythm	1
Ventricular ectopics	67
Ventricular tachycardia	7
Primary ventricular fibrillation	0
A-V block type I	35
A-V block type II	9
A-V block type III	7
Bundle branch block (sin – dx)	31
Asystole	6
No arrhythmia recorded	5

Relation between the transaminase values (GOT) among survivors and nonsurvivors

On an average the highest GOT value among surviving patients was 198 U (range 40–500) and among nonsurvivors 264 U (39–1040) a difference of 66 U $P<0.01$ calculated according to the usual methods

DISCUSSION

The mortality in our series (37 7 %) is high compared with that of other CC units (2 3 5 7 8 10 12 13 14 15 17 18) We consider that this difference is attributable to several factors such as selection of cases time interval between onset of symptoms and admission and the number of infarctions each patient has had

Selection

In order to be able to compare the mortality rate in different series it is important to state very carefully any principles of selection applied It is usually claimed that no form of selection has been employed but closer inspection of previously published articles or in some cases discussion with the author has often shown some form of

Table VII *Complications among 119 survivors*

	Pain only	Left vent failure	Cardiog. shock	Vasovagal shock	Ventricular fibrillation	Total
1st time myocardial infarction	88	11	0	3	1	103
2nd time myocardial infarction	15	1	0	0	0	16

selection In Lawrie s (10) series there are no patients over 70 as these are not treated in the CCU on the grounds that one can do more for patients under 70 This form of selection does not appear to us to be fully justified In our series age does not seem to influence the mortality to the extent one would expect In the under 70 group (113 cases) mortality was 32% and 46% in the over 70 group (78 cases)

The prognosis following resuscitation does not seem to be significantly influenced by the age factor (1 11)

Our mortality figures are weighted by nine patients who were admitted in a state of circulatory arrest and died usually within 24 hours following initially successful attempts to restore the circulation In other series (12 17) these cases are not included On the other hand we have excluded patients dead on admission or who died in the casualty department and were resistant to all forms of therapy In order to obtain comparable figures the WHO criteria for myocardial infarction should be followed and a postmortem examination should be made In previously published series the frequency of postmortem examinations as been too low or else not stated (12 17)

Interval between onset of symptoms and admission

Our hospital is situated in a relatively thinly populated area and for some patients the distance to the hospital may be as much as 60 km This is probably why most patients (53%) were admitted more than 6 hours after the onset of symptoms How this affects the mortality in our series is difficult to determine

Number of previous infarctions

In addition to the patient s condition on admission the number of previous infarctions is of great prognostic importance The frequency of serious complications (ventricular fibrillation asystole progressive myocardial insufficiency) increases with the number of previous infarctions and the difference in mortality in the three groups is most marked

Influence of complications on mortality

If one compares the state on admission of the survivors and nonsurvivors it is apparent that as expected complications are more frequent and more serious among the nonsurvivors 36 patients (50%) Nine of them had ventricular fibrillation seven had cardiogenic shock and twenty had cardiogenic shock and/or pulmonary oedema Among the survivors only 14 (11%) had complications (1 asystole 1 cardiogenic shock and 12 pulmonary oedema) The mortality in our series is high compared with that of the other series mentioned above We have tried to analyse the cause of death in each individual patient (Tables VI B and C) in order to ascertain whether with different methods of observation and treatment we might have been able to save any other patients Strictly speaking all patients die in a state of cardiac asystole We think that it is more profitable however to determine the way in which deterioration initially occurred and to try to find the cause However our detection of cardiac arrhythmia has not been entirely satisfactory based as it is on personal observation and a bedside-oscilloscope with a threshold alarm Of the 72 nonsurvivors 54% died within 48 hours and 78% within five days In general we kept the patients in the CCU for five days or longer if complications arose Would a longer period of observation have saved any other patients? Only four patients who were considered out of danger and had been transferred to another ward died in hospital following transfer One died from a cardiac rupture in the other patients the immediate cause of death is uncertain Progressive myocardial failure has been the cause of death in 62 5% of the patients For this condition there is at present no established form of therapy which with any certainty can influence the mortality One can possibly hope for improved results from the combined use of alpha receptor blockers and plasma expanders or with mechanical aids such as intra aortic balloon pumping (9)

Other causes of initial deterioration have been ventricular fibrillation in 19 cases (26%) and asystole in five (7%) Included in the group with ventricular fibrillation are nine patients who were in a state of circulatory arrest on admission We do not believe that any other form of treatment would have enabled these patients to survive as their condition was more or less hopeless from the outset The remaining ten developed primary ventricular fibrillation after admission One of these died later of a rupture three of brain dam

are, two of progressive myocardial insufficiency four of cardiac standstill The possibility can not be excluded that some of these might have survived if prevention of arrhythmias had been more adequate One of the four patients clinically diagnosed as dying from asystole was found to have a rupture We are therefore of the opinion that the question whether better diagnosis and therapy could have influenced the outcome can arise in 14 cases in our series Assuming that all 14 would have recovered the mortality rate would have b en reduced from 37 7% to 31% which is still a relatively high figure The group with cardiac rupture is unusually large and one wonders if this is because a postmortem examina tion was carried out in so many cases It is re markable that all the patients in the group with cardiac rupture were suffering from their first infarction and had been free from complications until the final period of deterioration commenced All died within five days four during the first twenty four hours

The total death rate from cardiac infarction is still unknown as many cases are not diagnosed for certain because death occurs outside the hospital Comparison between countries is often difficult because the frequency of admission to hospital varies widely

Some considerations regarding organisation and equipment

As a patient with a myocardial infarction is dangerously ill and there is great risk of com plications it is important that all patients with suspected infarction receive adequate supervision as soon as possible after onset of symptoms In Sweden we do not have the necessary staff to be able to follow the Belfast method (19) where the patients are not taken to hospital until an ECG apparatus is connected and a defibrillator is avail able Instead we must rely on improved informa tion in order that the patient can come under ob servation as quickly as possible after the onset of symptoms

Ambulance men must be able to warn the hospital via a special alarm system that a patient with suspected infarction is on the way to hospital so that a physician can be available immediately the patient arrives The CCU should if possible be situated near the admission department Un fortunately this could not be arranged in our case Where the patient has to be transported long distances inside the hospital trolleys can be equip ped with oscilloscopes and defibrillators At the outset we considered continuous ECG recording unnecessary now we know that it is necessary though it is still impractical time consuming and expensive The memory loop system is not ideal as the frequency of false alarms is very high probably about 90% according to Shillingford (personal communication) We intend in the first place to supplement our equipment with large centrally situated multi-channel slave oscilloscopes and thus increase the possibility of earlier and more effective recording of ventricular extra systoles There is at present no reliable system for graphic recording in association with an alarm system Routine chest roentgenographs are taken and we think that this examination is of value among other things in confirming suspected car diac failure

From the outset the staff was chosen with great care and it is worth pointing out that only one out of seven nurses has left the department during the time it has been in operation (nearly two years) The nurses have received special instruction by means of lectures and this has enabled them to undertake more exacting work The aim has been to enable them to apply many of the procedures normally applied by the physicians Now we are in the enviable position of having a staff of well trained nurses who can interpret ECGs and when a circulatory arrest occurs can immediately begin treatment with defibrillation and other procedures We consider this of great importance as having to wait for a physician implies a less favourable prognosis for the patient Several cases have been dealt with successfully before the physician ar rived In order to increase knowledge and stim ulate interest the nurses even make ward rounds interpret ECGs and suggest methods of treatment under the supervision and on the responsibility of a physician

To begin with we believed that the death rate from infarction could be reduced by immediate treatment of circulatory arrest Now we maintain as do Lown and others that the prevention of arrhythmias by administration among other agents of lidocaine (6 12) can to a great extent preclude the development of dangerous arrhythmias Dur ing the last five months when we have con sistently employed arrhythmia prophylaxis pri

marily with lidocaine we have experienced only one case of completely unexpected circulatory arrest To date we have mainly concerned ourselves with the treatment of circulatory arrest and the prevention of arrhythmias This has led to a reduction in mortality and a further reduction may result from improved knowledge of how best to treat shock of cardiac origin

Addendum Our series has been enlarged since the manuscript was submitted for publication and now consists of 288 patients representing a period of two years (September 1966–August 1968) Of these 288 patients 96 (33 3%) have died During 1968 we have carried out arrhythmia prophylaxis according to Lown et al (12) more uniformly and all patients have had breathing exercises led by a physiotherapeutist If the mortality rate during this period is compared with the earlier period the mortality has been decreased by more than a third from 39.2% to 25 9% This difference is statistically significant (*P* 0 05) A progressive fall in mortality is supposed by Julian in a recent article in Ann intern Med (69 607 1968) to be the most convincing piece of evidence to support the value of coronary care units rather than the exact figures of mortality We believe that the decrease in mortality rate is caused above all by the arrhythmia prophylaxis and the increased experience among all categories of the staff The breathing exercises may possibly also contribute to the decrease in mortality The mortality is however still ...vely high, probably because the series consists ... unselected patients

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VIBRATORY PERCEPTION AND BLOOD FLOW IN THE FEET OF DIABETICS

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Abstract The vibratory perception threshold has been measured in th feet of 25 diabetics and 16 non-diabetics The vascular response to artificially induced ischaemia ie the peak flow in the foot, was studied in the same patients by means of venous-occlusion plethysmography The following results were obtained

1. The vibratory perception threshold increased with increasing duration of diabetes as already shown by other authors.
2. The peak flow was often reduced in long term diabetic patients. This abnormality was associated with the presence of arterial calcification
3. No association could be demonstrated in individual diabetic patients between the vibratory perception threshold and the peak flow This finding suggests that ischaemia did not contribute to any major extent to the development of this nervous system abnormality

The significance of vascular factors for the development of diabetic neuropathy is still unsettled The concept that diabetic neuropathy might be due to isch emia was originally introduced by Woltman and Wilder (31) and renewed and supported by the work of Fagerberg (10)

However despite a considerable amount of work the results obtained from studying the relationship between the morphological change in nerves and vessels have not been consistent.

The purpose of the present study was to elucidate the same problem from a functional point of view This was done by measuring the vibratory perception threshold and the vascular response to artificially induced ischaemia in the feet of diabetics

METHODS

The blood flow in the foot was measured by venous occlusion plethysmography

The foot plethysm gra h u a had a general design similar by Stead and Kunkel (8 therefore not be report d in d T at the upper surface of the connected with the registra area of 21 cm

The plethysmograph was w t temperature thermostatically c r (3) An ink writin pi ter recorc r tion

The opening of th pleth smogr r limb passed was clo d by a thick rubber diaphragm. The thin diaphragm rubber cuff which was cement d to th to the malleoli Th diaphragm were p opening of the plethysmo raph b ar diaphragms and the cuff were mad inci base of a plaster-of Paris bandage c un just proximal to the malleoli

The calibration was done durin arrest cf u tion by injecting two ml of water in su cessi p nd measuring the mean rise of the pen

The volume of the enclosed foot was obtain d by recording the amount of water in the p ethysmogr ph at the end of the experiment and subtracting th n ount from the known volume of the apparatus.

A blood pressure cuff 6 cm wide was applied to the leg just above the ankle and a pressure of 50 mm Hg was used for venous occlusion (5)

A quantitative estimate of somatic nervous function was obtained by m asuring the vibratory perception threshold in the big toe The vibrat ry perception threshold was measured by a standardized technique (30) with a biothesiometer (Bio-Medical Instrument Co Chagrin Falls Ohio) and expressed in volts. Other procedures included palpation of the dorsal pedal artery and the posterior tibial artery and measurement of blood pressure

X ray films of the foot (dorsal plantar and lateral films) were taken by the specific technique for demonstrating arterial calcification

The patients were studied in a qu et room. The temperature of the room was controlled at 23 C (range – – 24 C) The blood flow was measured while the patient

Table 1 *Age (years) duration of diabetes (years) resting blood flow (ml per 100 ml foot per min) peak flow (ml per 100 ml foot per min) and vibratory perception threshold (volt) in the diabetic and non diabetic group of patients*

⁄ denotes the presence of severe cal ifi ation

Pat no	Age	Duration	Resting blood flow	Peak flow	Vibratory perception threshold	Severe calcification
Diabetics (duration less than 20 years)						
4	27	0	2.5	8 4	5	
6	27	8	5 4	9 0	40	
13	40	13	3 6	12 3	9	
14	28	4	2 9	10 5	10	
17	31	11	2 1	13 2	20	
20	28	16	4 9	7 6	13	
21	38	6	2 9	11 9	11	
24	35	14	5 0	13 7	46	
29	42	19	4 5	6 0	47	
33	42	1[illegible]	3 9	8 4	15	
34	48	6	4 6	8 6	[illegible]0	
37	41	9	4 7	11 1	13	
40	36	16	4 0	7 1	14	
Diabetics (duration more than 20 years)						
1	38	24	2 3	10 2	40	
2	37	35	3 0	0 6	50	×
5	33	26	3 3	8 4	43	
9	29	28	3 1	5 2	28	^
15	40	27	4 7	4 4	47	×
16	37	22	4 8	11 4	13	
18	42	21	3 6	2 8	23	×
23	43	40	6 7	3 2	12	
32	38	28	1 9	8 4	23	×
36	3[illegible]	[illegible]7	2 3	3 4	41	×
38	40	2[illegible]	5 5	3 9	17	×
	38	36	6 3	4 6	13	×
Non diabetics						
3	26		3 7	10 4	4	
7	36		4 1	10 7	12	
8	33		3 1	9 9	8	
10	37		4 0	9 2	9	
11	30		4 7	8 4	10	
12	9		2 1	10 1	8	
19	32		2 7	9 4	10	
22	41		3 6	10 1	9	
25	41		3 8	9 2	11	
26	46		2 9	13 2	12	
27	42		4 0	13 7	8	
28	44		2 8	9 3	18	
30	43		6 5	11 6	13	
31	43		3 7	9 4	14	
35	40		2 2	10 8	11	
39	42		4 8	10 8	10	

was resting comfortably in the horizontal position covered by two blankets The patients were at rest with the foot enclosed in the plethysmograph for at least 30 min before blood flow measurements were started

Blood flow measurements were performed in the following manner The resting blood flow was measured as quickly as possible approximately 30 times This procedure lasted about 8 min The circulation in the foot was then stopped by inflating the blood pressure cuff with a pressure of 300 mm Hg The arterial occlusion was maintained for 6 min and then suddenly released

The blood flow was measured as often as possible during the first minute and approximately 4 times every min until 9 min had elapsed by which time the blood

flow had returned to preocclusion level The whole procedure was then repeated until 4 series of resting blood flow measurements and 3–4 series of flow values after arterial occlusion had been obtained The investigation lasted for about 3 h and included approximately 250 blood flow determinations in each patient

The mean blood flow was calculated for each series of resting blood flow measurements and the mean of the four series was used in the analysis presented in the following A detailed analysis of the spontaneous variability of the resting blood flow in the feet has been presented elsewhere (6) and will not be considered here

The flow values obtained during reactive hyperaemia were plotted into a coordinate system, where the shape of the hyperaemic curve could be studied and its size measured Four parameters were calculated

(a) The maximal blood flow obtained during the hyperaemic period. This occurred generally within the first minute after the arterial occlusion was released

(b) The peak flow which is obtained by subtracting from the maximal blood flow the resting blood flow measured in the 8 min period immediately before the arterial occlusion

(c) The first minute flow or area. This was measured with a planimeter

(d) The first minute excess flow which was calculated by subtracting the mean resting flow from the first minute flow

The mean results of the three or four series obtained were used in the analysis presented in the following.

Conventional probability levels of significance have been used in the statistical analysis, a p value greater than 0 05 not being considered significant.

MATERIAL

Forty-one individuals were studied Twenty five were diabetics, 15 males and 10 females The mean age was 36 years (range 27–48 years) The duration of diabetes ranged between 0–40 years and was more than 20 years in approximately half of the diabetic patients One fourth of the patients had clinical neuropathy in the sense of pain and paraesthesia Sixteen individuals were non diabetics. None of them had symptoms of diabetes mellitus or glucosuria The mean age was 38 years (range 6–46 years) Ten were males and six females Diabetics and non-diabetics were examined at random.

RESULTS

All important data are summarized in Table 1

Vibratory perception threshold

The vibratory perception threshold measured in the big toe increases as is well known (7 10 15 30) with increasing duration of diabetes To obtain linearity and constant standard deviation for regression analysis the results from the examination of the threshold were converted according to the following equation $z = 100 \log$ threshold The transformation used elsewhere (7) was found to be less satisfactory for the present data

The equation of the regression line is

$$y (z) = 1\ 08x + 110\ 8$$

and the regression coefficient differs significantly from zero ($p < 0\ 05$) The vibratory perception threshold could not be shown to be associated with the age of the patients in the present small series The mean threshold in the non-diabetic group is 10 and in the diabetic group 25 a significant difference ($p < 0\ 001$ Mann Whitney U test)



Fig 1 Blood flow (ml per 100 ml foot per min) obtained after release of arterial occlusion (reactive hyperaemia) plotted against time Results obtained in a non-diabetic are shown (case 8)

Resting blood flow

In the non diabetic group the mean resting blood flow averages 3 7 ml per 100 ml foot per min $\pm 1\ 1$ (range 2 1–6 5 ml) No association with age could be demonstrated

In the diabetic group the mean resting blood flow averages 3 9 ml per 100 ml foot per min $\pm 1\ 3$ (range 1 9–6 7 ml) The resting blood flow in the diabetic group shows no association with the age of the patient, the duration of the disease the vibratory perception threshold or the peak flow

Neither the mean blood flow nor the range in the two groups differ significantly from each other

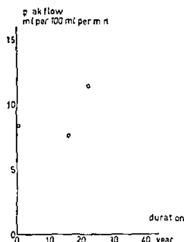


Fig 2 Peak flow (ml per 100 ml per min) obtained in the 25 diabetics plotted against the duration of the disease The symbol (●) denotes the presence of severe arterial calcification

Blood flow response during reactive hyperaemia

Fig 1 shows an example of the time course of the reactive hyperaemia obtained in a non diabetic (case 8) The blood flow is high immediately after release of the arterial occlusion and then rapidly decreases towards basal levels

In the control group the maximal blood flow ranges between 12 1 and 19 3 ml per 100 ml per min and averages 14 1 ± 2 1 ml

The maximal blood flow increases with increasing mean resting blood flow The equation of the regression line is

$$y = 1\ 12x + 9\ 96 \quad (p < 0\ 002)$$

The peak flow which was calculated by subtracting the mean resting blood flow from the maximal flow averages 10 4 ml ± 1 4 and ranges between 8 4 and 13 7 ml per 100 ml foot per min The peak flow is independent of the resting blood flow ($p > 0\ 1$), as the regression coefficient above is close to one For this reason the peak flow was used in the analysis presented below

The first minute flow is also a function of the mean resting blood flow ($p < 0\ 001$) while the first minute excess flow is independent of the mean resting blood flow ($p > 0\ 1$)

In the diabetic group the peak flow ranges between 0 6–13 7 ml per 100 ml foot per min and averages 7 8 ml ± 3 6 ml Considering only the patients with a duration of the disease less than 20 years the peak flow is of the same order of magnitude as in the non diabetic group ($p > 0\ 1$) However when the disease has lasted for 20 years or more the peak flow is often considerably reduced Fig 2 shows the relationship between the peak flow and the duration of the disease Regression analysis was carried out with duration of the disease and age of the patient as the independent variables and the peak flow as the variable However only the first factor the duration of diabetes is of importance ($p < 0\ 001$) The equation of the regression line is

$$y = -0\ 223x + 12\ 0$$

The same general results were obtained when the first minute excess flow was considered ($p < 0\ 002$ for an association between the excess flow and the duration of the disease) There is a strong association in individual patients between the peak flow and the first minute excess flow ($p < 0\ 001$)

The mean peak flow and the first minute excess flow differ significantly from the corresponding means in the control group ($p < 0\ 01$ – $0\ 01$)

The relationship between the vibratory perception threshold and the peak flow

In Fig 3 the vibratory perception threshold has been plotted as a function of the peak flow It is seen that all possible combinations are represented Patients with a high peak flow may have a low normal threshold or a high abnormal threshold Patients with a low and pathological peak flow may have an increased or a normal threshold Thus information about the presence or absence of the nervous system abnormality studied cannot be obtained from knowledge of the blood flow response to artificially induced ischaemia ($p > 0\ 1$)

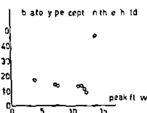


Fig 3 The vibratory perception threshold plotted against the peak flow Results obtained in the diabetic group of patients are shown

The same results are obtained when the relationship between the first minute excess flow and the vibratory perception threshold is studied No association is obtained ($p > 0.1$)

The time course of the reactive hyperaemia in patients with a high threshold and a peak flow within normal limits shows no striking difference from the time course of the curves obtained in the non-diabetic group

Reproducibility of the peak flow and the vibratory perception threshold

The reproducibility of the peak flow in the individual persons the intra individual variance was studied by calculating the standard deviation of the three or four different peak flow values obtained in the single patient

The mean of the standard deviations of the peak flow averages 1 3 ml $\pm$ 0 7 in the non-diabetic group and 0 9 ml $\pm$ 0 4 in the diabetic patients with a mean peak flow higher than 8 0 ml and 0 8 ml $\pm$ 0 3 in the group with a peak flow less than 8 0 ml The evaluation of the vibratory perception threshold was based on three measurements in each single person The three measurements showed either identical values or deviated from each other by a few units of volts

Other Features

Pulsation of the dorsal pedal artery and the posterior tibial artery

Pulsation in the two arteries was absent in two diabetics (cases 2 and 36) and pulsation in the dorsal pedal artery could not be felt in one non diabetic (case 12) In all other cases strong pulsation was felt without difficulty in both arteries

Blood pressure

In the non diabetic group the mean systolic pressure averages 124 mm Hg $\pm$ 17 In the diabetic group the mean systolic pressure averages 140 mm Hg $\pm$ 24 This difference is significant ($p <$ 0 05) The diastolic pressure averages 80 mm Hg $\pm$ 11 in the non-diabetics and 87 mm Hg $\pm$ 13 in the diabetics This difference is not significant No association could be established between the blood pressures and the duration of diabetes

Calcification of the arteries

X ray films of the arteries of the foot were obtained in all diabetics except one (case 14) The results are given in Table I Extensive calcification i e calcification in several parts of the arterial system of the foot, has been denoted by an X In Fig 2 the symbol (●) indicates the presence of severe calcification Minor calcification was present in a few patients and was localized to a small area of one of the arteries The present study did not include a sufficient number of persons to allow an exact statistical analysis of the interrelationships between decreased peak flow the presence or absence of calcification of the arteries and the duration of diabetes This problem has been evaluated in another investigation (7) It appears however from the figure and the table that extensive calcification of the arteries is associated with a small peak flow and a low peak flow is associated with the presence of extensive calcification in all cases except one The results are thus in accordance with those presented elsewhere (7)

X ray examination was generally not performed in the non-diabetic group However in an X ray study of the arteries of the foot in 36 non diabetics 25 males (7) and 11 females (5) of comparable age it was found that only one person a man 32 years of age showed minor calcification in the first interdigital space

The type of calcification present in the arteries of the foot is calcification of the media in all cases (7 12 18)

Clinical neuropathy

Clinical neuropathy was present in cases 1 2 5 6 15 24 and 29 It appears from Table I that these patients all had a high vibratory perception threshold

DISCUSSION

The vascular response to ischaemia occurs in skin and muscles Arterio-venous anastomoses are apparently not involved (13) The response is mediated locally in the tissue concerned and the response is not dependent on the integrity of the sympathetic nervous system (2 9 14 17)

The observation presented here the decrease of the peak flow with increasing duration of diabetes mellitus is in accordance with the results presented elsewhere (7) demonstrating that the maximal blood flow in the anterior tibial muscle measured by radioactive xenon[133] is a

low in long term diabetics As the blood pressure was normal or slightly elevated in these patients the abnormality is due to an increased peripheral resistance

A rather peculiar phenomenon is the association between low peak flow as well as a low maximal blood flow (7) and the presence of arterial calcification of the media The presence of this type of calcification per se in non diabetic persons seems generally to be accepted as an innocent finding without any functional significance and occurring independent of intimal change (8 18 23 25 27) The established association in diabetic persons between medial calcification and decreased peak flow does suggest a somewhat different development of the peripheral vascular disease in diabetics and non diabetics although the calcification per se might contribute little to the increased peripheral resistance

There have been several other studies of the vascular function and nervous function in the feet of diabetics However from a purely vascular point of view the results published are difficult to interpret In several cases the vascular dilatation has been induced by indirect heating or by nerve blocking (1 4 21 22 26) Although interesting results may be achieved in this way it ot be known from this sort of stimulation a decreased vascular reponse is due to a scular lesion per se or to a pre existing autonomic neuropathy Abnormalities demonstrated in the way mentioned above should be called neuro vascular abnormalities and the pathophysiological mechanism which is probably involved has been briefly discussed elsewhere (6) It should also be noted that some of the methods of measuring blood flow used by the authors mentioned and by others (20 24) do not give a reliable estimate of the actual size of the blood flow e g identical cutaneous temperature does not necessarily indicate identical cutaneous blood flow (11)

It has been discussed for several years whether or not vascular factors are important for the development of various types of diabetic neuropathy The concept that diabetic neuropathy might be due to ischaemia was originally introduced by Woltman and Wilder (31) and renewed and supported by the work of Fagerberg (10) It has been shown in several studies (16 30) that a metabolic factor must also be operative In the present study it was not possible to establish any relationship in individual diabetic persons between the vascular response to ischaemia i e the peak flow and the vibratory perception threshold This lack of agreement between impaired nervous and vascular function suggests that ischaemia is not a major factor in the development of the nervous system abnormality studied This does not exclude the possibility that ischaemia contributes to the development of this abnormality in diabetic patients with a very severe vascular disease (29)

The present study does not include a sufficient number of patients with neurological symptoms to draw a conclusion about the significance of vascular factors for the development of clinical neuropathy (19)

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Book Review

Gammapathies infections cancer and immunity Edited by V Chini L Bonomo and C Sirtori 108 pages 52 ill Carlo Erba Foundation Milan 1968

At this symposium some very topical biological and clinical problems were treated and debated by specialists from many countries The results have now been published in an excellent edition in a series of monographs sponsored by the Carlo Erba Foundation To me it seemed excellent that persons came together both from the basic sciences immunology and biochemistry as well as clinicians with interests in autoimmunity and in immunoglobulin formation in the systemic diseases of immunocytes such as myeloma and macroglobulinemia

The clinician Bonomo who was one of the hosts in Bari had in collaboration with Dammacco studied the gamma globulin picture in dif
infectious diseases They were able to show
both in leprosy and in chronic respiratory
symptoms were found that we usually
nect with rheumatic disease or autoimmunity Marmont of Genoa gave a very interesting review of immunocytology with excellent illustrations (see coloured plate in the book) showing immunofluorescence of cells in different types of disease among other things he could demonstrate typical γA fluorescence in flaming plasma cells from myeloma patients The same was true mutatis mutandis of the lymphocyte like cells in the bone marrow from patients with cold agglutinin disease when they were exposed to fluorescent antimacroglobulin

More fundamental problems were discussed by Cioli who collaborates with one of the leading young medical investigators in Italy Baglioni They had analysed a large number of different Bence Jones proteins and were able to establish certain principles of importance for the understanding of their structure Their work is of great interest also for the clinician in charge of myeloma patients

Holborow gave a review of recent advances in the understanding of the previously so called autoimmune mice from New Zealand At the present moment it seems as if they were suffering rather from some transmissible virus-like disease that stimulates their production of polyclonal immunoglobulin

The reviewer discussed the possibility that a similar process may be at work also in human patients and summarized our experience regarding the two types of immunocyte proliferation monoclonal and polyclonal Several other topics were also treated It seems to me that the paper by Alexander on experimental tumours with lymphocyte injections from animals that had been immunized against the same tumour was of very great interest

Jan Waldenstrom Malmo

SUBJECT INDEX

(Supplements, see page XII)

Kidney

Liver

Lung

Medical history

Metabolism

Muscles

Tumours

Urinary tract

Vitamins

BOOK REVIEW

SUPPLEMENTS TO VOLUME 185

LIST OF AUTHORS